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LONG-TERM EFFECTS OF DIETARY COPPER SOURCE AND LEVEL ON PERFORMANCE AND HEALTH OF SOWS AND PIGLETS

DISSERTATION

A dissertation submitted in partial fulfillment of the
requirements for the degree of Doctor of Philosophy in the
College of Agriculture, Food and Environment
at the University of Kentucky

By

Ning Lu

Lexington, Kentucky

Director: Dr. Merlin D. Lindemann, Professor of Animal and Food Sciences

Lexington, Kentucky

2017

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ABSTRACT OF DISSERTATION

LONG-TERM EFFECTS OF DIETARY COPPER SOURCE AND LEVEL ON PERFORMANCE AND HEALTH OF SOWS AND PIGLETS

The objectives of this study were to investigate the long-term effects of feeding increasing supplemental levels (20, 120, or 220 mg/kg) of dietary copper (Cu) as tribasic copper chloride (TBCC) or copper sulfate ($CuSO_4$) on performance, antioxidant status, nutrient digestibility, and trace mineral deposition of sows and piglets; as well as to assess nursery dietary Cu levels on growth performance and response to immunological challenge in nursery pigs from sows fed either high or low Cu diets. In the long-term sow experiment, sows fed TBCC diets had greater adjusted weaning weight for litter and piglet ($P < 0.10$), as well as adjusted litter and piglet weight gain ($P < 0.10$) when compared to sows that received $CuSO_4$ diets. Increasing dietary Cu level linearly increased live born piglet weight ($P = 0.06$). Sows fed TBCC diets had lower apparent total tract digestibility (ATTD) of ether extract ($P = 0.01$) during late gestation, but greater ATTD of dry matter, nitrogen, and phosphorous during lactation ($P < 0.05$). Increasing Cu levels linearly increased dry matter digestibility in lactating sows ($P = 0.02$). Milk from sows fed TBCC diets had a greater concentration of protein ($P = 0.02$) than that from sows fed $CuSO_4$ diets. Increasing Cu levels increased levels of milk fat and Cu (linear, $P < 0.05$); but linearly decreased lactose and Zn levels ($P < 0.05$). Lactating sows fed TBCC diets had a greater activity of Cu/Zn superoxide dismutase (SOD) and ceruloplasmin in serum than those fed $CuSO_4$ diets ($P < 0.05$). Increasing dietary Cu levels increased total and Cu/Zn SOD activity for lactating sows (linear, $P < 0.05$). Sows fed TBCC diets had lower concentrations of Cu ($P = 0.04$), but higher concentrations of iron and manganese ($P < 0.05$) in the liver, when compared to those fed with $CuSO_4$ diets. In addition, liver Cu concentrations increased with increasing dietary Cu levels (linear and quadratic, $P < 0.05$). Increasing dietary Cu levels resulted in the elevation of concentrations and contents of Cu in the liver of weanling piglets (linear, $P < 0.0001$). In the nursery pig experiment, pigs from sows fed 120 mg/kg Cu diets had greater ADG from d 0 to 14 ($P < 0.05$), and tended to have greater ADG in the overall period ($P < 0.08$), when compared to pigs from sows fed 20 mg/kg Cu diets. During the lipopolysaccharide challenge period, the challenged pigs from sows fed 120 mg/kg Cu had a greater overall rectal temperature than those from sows fed 20 mg/kg Cu

(P = 0.01). Also, the challenged pigs fed with 220 mg/kg Cu diets had greater serum tumor necrosis factor-alpha concentration over time as compared to those fed 20 mg/kg Cu diets (P = 0.03). In summary, the TBCC may be a superior Cu source compared to $CuSO_4$ regarding reproductive performance, and higher dietary Cu levels result in greater birth weight of piglets; furthermore, high Cu levels in sow and nursery diets promote growth performance of nursery pigs and affects their responses to immunological challenge.

KEYWORDS: Sows, Nursery pigs, Tribasic Copper Chloride, Copper Sulfate, Reproductive Performance, Immunological challenge

Ning Lu

November 17, 2017

LONG-TERM EFFECTS OF DIETARY COPPER SOURCE AND LEVEL ON PERFORMANCE AND HEALTH OF SOWS AND PIGLETS

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Director of Graduate Studies

November 17, 2017

To my beloved family

especially to my super energetic daughter

without whom this work would have been completed much earlier

大恩不言谢，大爱溢于心

这两行只是为了

对称

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CHAPTER 1. Introduction

Sows are considered one of the most critical constituents of the swine industry because sow productivity determines the capacity of the swine herd, and genetic potential of the sows defines the maximum potential productivity of the entire system (Ball et al., 2008). Over the past 15 years, the number of pigs weaned per sow per year (PSY) has increased from 20.40 to 24.91 pigs in the US, and it is likely that the improvement of genetics and management may increase PSY up to 30 to 40 pigs in the future (PigCHAMP, 2016; Koketsu et al., 2017). However, greater sow productivity inevitably leads to increased mobilization of minerals in body reserve, and reproductive capacity can be compromised if the mineral needs for reproductive demands exceed body stores and dietary intake (Mahan, 1990). Mahan and Newton (1995) demonstrated that body mineral contents, which included calcium, phosphorous, magnesium, potassium, sodium, aluminum, zinc, and Cu, had significantly declined in sows that completed three parities, compared to those in similarly aged, nongravid gilts.

As one of the minerals that is being depleted in sow body storage with advancing parity, copper (Cu) is required to serve many biological roles in the body, such as supporting iron metabolism, protecting tissues from oxidative damage, and maintaining immunity. The latest edition of NRC estimated that the Cu requirement of growing pigs is 3 to 6 mg/kg and for gestating and lactating sows is 10 and 20 mg/kg (NRC, 2012). In addition to meeting a nutritional requirement, pharmacological concentrations of Cu (125 to 250 mg/kg) have been proven to enhance growth performance of growing pigs. Cromwell (2001) summarized 41 experiments conducted at the University of Kentucky and concluded that 200 to 250 mg/kg of supplemented Cu improved daily gain by 11.9 and 6.9%

during starter and grower phases, respectively. Dietary supplementation of pharmacological levels of Cu (250 mg/kg) in gestating and lactating diets from parity 1 to 6 has been demonstrated to increase Cu concentrations in sow liver and kidney, as well as improve piglet weight at birth and weaning (Cromwell et al., 1993). However, the effects of high dietary Cu from different sources on reproductive sows have not been assessed. In addition, the effects of high Cu in maternal diets on performance and immunity of progeny have not been reported.

Therefore, the objective of the present research was to determine the effects of dietary Cu sources and levels on performance and health of sows (Chapter 3), and the effects of dietary Cu levels on growth performance and response to immune challenge in nursery pigs from sows fed either high or low Cu (Chapter 4).

CHAPTER 2. Literature Review

2.1 Changes and challenges of sow production

It is believed that the domestication of pigs occurred about 9,000 years ago. Because of their adaptability to a wide variety of environmental conditions and feeds, and characteristics such as fast growth rate and relatively short reproductive cycle, pigs were made to become one of the most important food animal species for humans (Kim, 1999; Rothschild and Ruvinsky, 2010). The number of pig produced worldwide has increased from 400 to 900 million head per year from 1961 to 2008; and pork accounts for over 40% of all red meat consumption in the world (FAO, 2010). The United Nations has projected that global population will expand to 9 to 10 billion by 2050. Therefore, pork production is expected to grow to feed the growing population in the future.

Sows play an important role in fulfilling the demand for pork because their productivity determines the number of pigs that are available for pork production. Sow productivity is usually assessed according to the number of pigs weaned per litter, per year, or per lifetime. With continuous improvement in genetic selection, nutrition, and management during the past decades, sow productivity has improved dramatically. China, the European Union, and the US are the major pork producing countries in the world; they accounted for 80% of total pork production globally in 2016 (USDA Foreign Agricultural Service, 2017). Sow productivity of these countries has increased by 11 to 28% from 2001 to 2013, regarding weaned or finished pigs produced per sow per year (Table 2.1). Consequently, greater sow productivity leads to more nutrients being required for the development of more and/or heavier fetuses during gestation, as well as to produce a greater amount of milk to meet the demands of the larger litter during lactation (Kim et al., 2013).

Table 2.1. Sow productivity improvement (pigs per sow per year) of major pork producing countries

	Year				
Country	2001	2004	2007	2010	2013
China[1]	12.59	13.42	13.35	13.74	13.94
EU-27[2]	17.30	18.10	18.61	20.41	22.10
US[3]	19.70	21.25	22.40	23.30	24.88

[1]Adapted from China Animal Husbandry Yearbook 2002 to 2014. Numbers represent finished pigs per sow per year.
[2]Cited from AHDB PORK (2016). Numbers represent finished pigs per sow per year.
[3]Cited from Pig Champ (2016). Numbers represent weaned pigs per sow per year.

Improvement of sow productivity is associated with changes in nutrient requirements, which are reflected in NRC publications over the past 2 decades (Table 2.2 and 2.3). Nutrient requirements of gestating sows were estimated for multiple stages in NRC (2012), and sows in the latter part of gestation (after day 90 of gestation) require a much greater amount of energy and nutrients (lysine, calcium, and phosphorus) than in previous NRC editions. Nutrient requirement estimates for lactating sows also increased in NRC (2012) compared to NRC (1988, 1998), which reflects the greater demands of nutrients for increased milk production. Regarding trace minerals, the requirement estimates of Cu, manganese (Mn), and zinc (Zn) in NRC (2012) increased at least 40% compared to the previous NRC editions. However, the requirement estimate of iron (Fe) did not change dramatically over the past 2 decades.

Even though nutrient requirements for sows have increased to meet the elevating demands of increased production, a conflict still exists between the selections for greater productivity and the target of a prolonged productive lifetime. Prolonged productive lifetime is considered beneficial to pig producers because of fewer unproductive days, greater acquired immunity to herd disease, and lower replacement costs (Lucia et al., 2000a; Hoge and Bates, 2011). However, the intensive selection in the breeding herd has led to

Table 2.2. Comparison of NRC nutrient requirement estimates of gestating sows

Item	NRC 1988	NRC 1998	NRC 2012	
Breeding weight, kg	-	125 to 200	140 to 205	
Anticipated gestation weight gain, kg	-	55 to 35	65 to 45	
Days of gestation	-	-	< 90	> 90
Feed intake, kg/d	1.90	1.80 to 1.96	2.05 to 2.21	2.45 to 2.61
Requirement, amount/day				
ME, Mcal	6.1	6.0 to 6.4	6.4 to 6.9	7.7 to 8.2
Lysine, g[1]	8.2	9.4 to 11.4	7.7 to 12.4	13.1 to 19.3
Calcium, g	14.2	13.9	8.9 to 12.4	16.4 to 19.9
Phosphorus, g[2]	11.4	11.1	7.7 to 9.9	12.5 to 14.8
Copper, mg	9.5	9.3	21	
Iron, mg	152	148	168	
Manganese, mg	19	37	52	
Zinc, mg	95	93	210	

[1]Lysine requirement is presented on the total basis.
[2]Phosphorus requirement was presented on the total basis.

Table 2.3. Comparison of NRC nutrient requirement estimates of lactating sows

Item	NRC 1988	NRC 1998	NRC 2012	
Post-farrowing weight, kg	-	175	175	210
Anticipated daily weight gain of nursing pigs, g/d	-	150 to 250	190 to 270	
Feed intake, kg/d	5.30	4.31 to 6.40	5.93 to 5.95	6.61
Requirement, amount/day				
ME, Mcal	17.0	14.1 to 20.9	18.7	20.7
Lysine, g[1]	31.8	35.3 to 61.9	48.7 to 56.5	52.4 to 60.5
Calcium, g	39.8	39.4	35.3 to 45.0	37.7 to 48.1
Phosphorus, g[2]	31.8	31.5	17.7 to 22.6	18.9 to 24.0
Copper, mg	26.5	26.3	119.3	
Iron, mg	424	420	447	
Manganese, mg	53	105	149	
Zinc, mg	265	263	597	

[1]Lysine requirement is presented on the total basis.
[2]Phosphorus requirement was presented on the total basis.

high culling rates within commercial herds between 40 to 50%, as well as increased sow death rate from 6.5 to 10.0% during the past 15 years in the US (Figure 2.1). Lucia et al. (2000b) reported that reproductive females finish 3.3 parities on average before being removed from breeding herds in the US. A sow culled at early parities obviously produces fewer pigs in her lifetime, compared to sows that stay in the breeding herd for a longer period (Stalder et al., 2004). Theoretically, a sow must remain in the herd at least for 3 parities to produce enough piglets to pay for herself (Stalder et al., 2000). Therefore, it is important that the management practices and nutritional strategies of commercial producers aim to improve sow longevity for better profitability and breeding herd efficiency (Thingnes et al., 2015b).

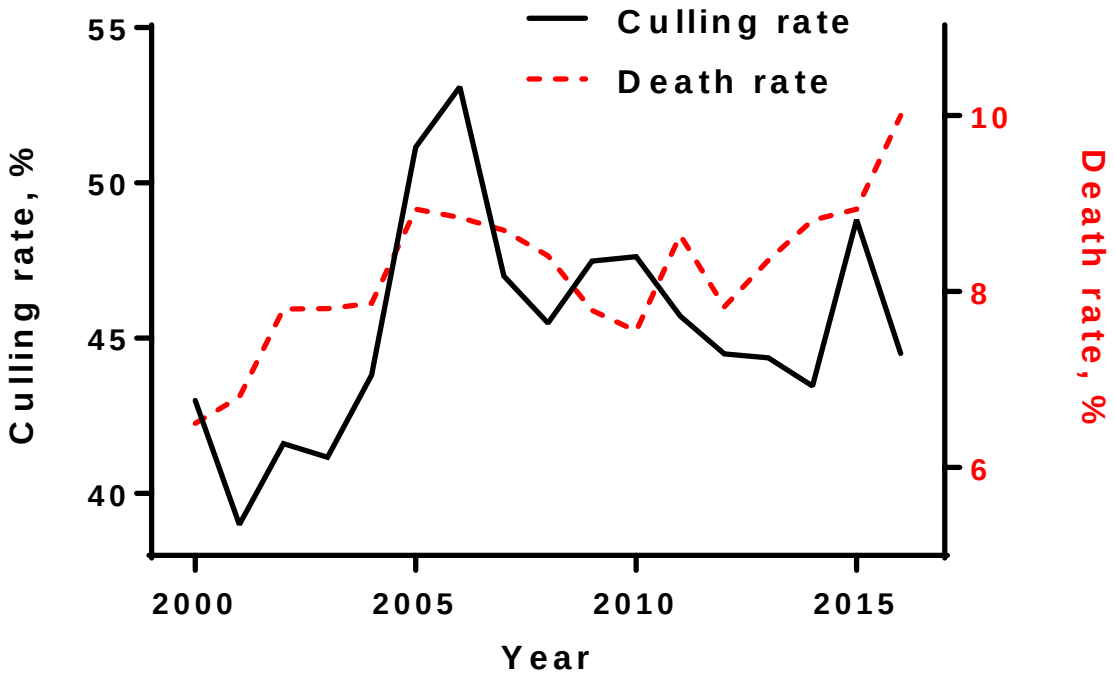

Figure 2.1. Culling rate and sow death rate in the US from 2000-2016 (Adapted from PigChamp, 2016, 2017)

2.2 Sow longevity

2.2.1 Longevity and productivity

Sow longevity is a concept that refers to the length of a productive lifetime of sows, which can be evaluated by various indices including productive lifespan, culling rate, and replacement rate (D'Allaire et al., 1992; Abell, 2013). Sow longevity has been defined differently among studies, which included the length of productive life and lifetime prolificacy (Serenius and Stalder, 2004), as pigs born alive per day of life (Holder et al., 1995), or as the length of productive life (Tarrés et al., 2006b).

The increasing sow culling rate and death rate over the past 15 years (Figure 2.1) may suggest a declined sow longevity, which is detrimental to the efficiency and profitability of pig production (Díaz et al., 2015). Mote et al. (2009) investigated the difference in reproductive performance between that of gilts and that of sows that already had produced a minimum of 5 litters for the subsequent 4 continuous parities. The results showed that the older sows had significantly more pigs born alive ($P < 0.05$) during the first 3 parities, and still had numerical superiority in the fourth parity. The declined sow longevity is driving the parity distribution of a breeding herd toward a greater percentage of gilts, which results in higher gilt replacement cost and lower productivity. Also, King et al. (1998) concluded that the average number of non-productive days increases by 2.6 days as the percentage of gilts inventory in the breeding herd increases by 1%. Moreover, due to the immature immune system of gilts when they are introduced to breeding herds, they are more likely to get infections from the pathogens that already exist among the reproductive females (Sanz et al., 2002). The higher risk of disease for gilts may negatively affect their reproductive performance and even further shorten the longevity of the breeding herd.

2.2.2 Reasons for sow loss

Sow longevity is primarily determined by how long they could stay in in the breeding herds. There are numerous reasons for removing sows from breeding herds. Table 2.4 provides a listing of the most common reasons for removing sows, which was summarized from 19 studies by Stalder et al. (2004). It is evident that reproductive failure, which includes no postweaning estrus, inability to conceive, and failure to farrow, is the predominant reason for removal of sows from the breeding herd. Sows that were removed for reproductive failure have fewer piglets born alive per year and more non-productive days compared to those culled for other reasons (Sasaki and Koketsu, 2011). Mote et al. (2009) pointed out that reproductive failure was the predominant reason for removal of younger sows (produced less than 5 litters); but not for older sows (produced a minimal of 5 litters).

The term “Old age” is a relatively subjective reason, and its definition can be very different between breeding herds because pig producers can make their own decisions on when to cull the old sows (D'Allaire et al., 1987). However, old age is not frequently used for removing sows before parity 5 but is the predominant reason for sows older than parity 5 (Mote et al., 2009; Díaz et al., 2015).

Poor performance includes small litter size at farrowing and weaning, low piglet birth/weaning weight, and poor mothering ability (Díaz et al., 2015). Poor performance is not a common reason for culling young females because they are allowed to express reproductive capabilities before parity 3 or 4. However, the prevalence of poor performance as a removal reasons starts to increase as sows mature and becomes one of

the most common reasons for females having 4 to 6 parities (D'Allaire et al., 1987; Lucia et al., 2000b).

Table 2.4. Summary of reasons for sow removal[1]

Item	Average, %[2]	Range, %
Reproductive failure	28.5	8.8 to 39.2
Old age	16.7	2.2 to 33.4
Poor performance	12.4	1.0 to 22.4
Feet, legs, and locomotion disorders	10.9	6.1 to 15.0
Death	7.1	3.0 to 12.3
Milking problems	6.1	0.9 to 13.0
Injury, health, and disease	4.1	0.8 to 13.3
Farrowing problems	3.2	1.6 to 7.2

[1]Adapted from Stalder et al. (2004).
[2]Average of results from 19 published studies.

Lameness is one of the most important reasons for the premature culling of sows; and several factors could cause lameness in breeding herds, such as housing, genetics, and nutrition (Bradley, 2010; Díaz et al., 2015). Joint arthritis and osteochondrosis are the first and second primary reasons for the euthanasia of lame sows, respectively (Bradley, 2010). As a result of culling young sows due to lameness, the average longevity of breeding herd is compromised (D'Allaire et al., 1987; Lucia et al., 2000b; Pluym et al., 2011).

Mortality also makes up a considerable portion of sow removals in breeding herds. Sow deaths, as a percentage of total breeding herd females, has increased steadily from 6.5 to 10.0% during the past 15 years (Figure 2.1). The common reasons for sow mortality are associated with sow ages; it appears that the deaths of older sows are more likely to be caused by heart failure, torsion of the gut, cystitis, and uterine prolapse. In contrast, arthritis, endometritis, pneumonia, locomotion problems, and ulcers are more problematic for gilts and young sows (Chagnon et al., 1991; Stalder et al., 2004).

2.2.3 Nutritional strategies and sow longevity

The genetic selection of breeding herds over the past decades has successfully reduced body fatness as well as improved feed efficiency and lean tissue growth of finishing pigs (O'Dowd et al., 1997). However, an unfortunate conflict existed between the selection for leaner animals and the requirement of maintaining high productivity of sows through successive parities (Thingnes et al., 2015b). Stalder et al. (2004) reported that the replacement rate in commercial herds was close to 50% worldwide; which suggested that the current nutritional strategies might not be capable of sustaining high production over various parities for prolific modern sows. Thus, the nutritional strategies for breeding herds need to be designed with considerations of improving sow longevity.

2.2.3.1 Feeding level

With the purpose of achieving a long, productive life, developing gilts need to build up adequate body reserve that can be utilized for the subsequent reproductive cycles. Body composition (backfat depth and BW) of gilts at first service was thought to affect how long the gilt could stay in the breeding herd (Díaz et al., 2015). It was reported that gilts with a backfat thickness greater than 18 mm were more likely to stay in the breeding herd for at least 4 parities ($P < 0.05$) compared with the lean gilts with 10 mm or less backfat thickness (Brisbane and Chenais, 1996). Tarrés et al. (2006a) pointed out that gilts with a backfat thickness less than 16 mm at the end of the growth period had a significantly higher risk to be culled after the third farrowing ($P < 0.05$). However, the effects of body condition at first mating on subsequent reproductive performance and longevity of sows are conflicting. Jorgensen and Sorensen (1998) fed gilts from 6 weeks of age to mating with 3 feeding regimes, which included Semi ad libitum (fed ad libitum until 10 weeks of age and then ad

libitum for 30 min at 2 daily feedings thereafter), Control (feeding scale according to the Danish standard), and 75% of Control. The results of the subsequent 4 reproductive cycles showed that there was no significant difference in litter size and weight, milk yield among the feeding levels. Sørensen et al. (1998) used the same set of animals for culling reason analysis, and the results showed that Semi ad libitum fed sows had the poorest leg weakness score, causing a significantly greater culling rate and younger age at culling when compared to the other feeding levels ($P < 0.05$).

Gestation feeding regimes are designed to supply adequate nutrients for fetal and associated uterine tissue growth, mammary gland development, and maternal BW gain. Regarding gilts, increasing the feeding level during the entire gestation period led to greater gestational BW gain and depressed lactation feed intake, which thus resulted in poor reproductive performance that might increase the risk of being culled (Dourmad, 1991; Weldon et al., 1994). However, Mahan (1998) reported that increased gestation feed intake by 6.0 to 7.2% during parity 1 to 5 resulted in larger gestation BW gain and litter size ($P < 0.01$), but did not affect lactation feed intake or BW change. The discrepancy might suggest that the additional gestation BW gain due to higher feed intake may affect the lactation feed intake and performance of gilts but not sows.

The first month of gestation is critical for sows since this is the period in which pregnancy is established and litter size is determined (Langendijk, 2015). It is believed that high feeding levels during the early stage of gestation lead to declined embryo survival rate because a greater amount of feed reduces systemic concentrations of progesterone, which acts on the uterus to improve the uterine environment in preparation for implantation (Prime and Symonds, 1993; Jindal et al., 1996). However, there have been some studies

that compared a high vs. control feeding levels throughout the first 3 weeks of gestation that reported embryo survival was not affected by feeding levels (Toplis et al., 1983; Quesnel et al., 2010; Condous et al., 2014). Hoving et al. (2011) reported that increased feed allowance by 30% from day 3 to 32 after the first insemination increased BW gain and backfat thickness at the end of the current reproductive cycle ($P < 0.05$), improved litter size (total number of born and number of born alive, $P < 0.05$), but did not affect farrowing rate (proportion of females served that farrow) in the subsequent parity.

Fetal growth rate increases dramatically during the last trimester of gestation compared to early and mid-gestation (Ma, 2011). A constant feeding level throughout the entire gestation period may not provide adequate nutrients for sow and fetuses during late gestation and results in catabolism of maternal tissue (Shields and Mahan, 1983; Trottier, 1991). Cromwell et al. (1989) reported that increased feed intake by 1.36 kg/d on top of gestation feed allowance (1.82 kg/d from March through November and 2.27 kg/d from December through February) from day 90 of pregnancy to parturition significantly increased sow BW gain from day 90 to 110 of gestation ($P < 0.001$), number of live piglets at farrowing ($P = 0.06$) and 21 day postpartum ($P = 0.09$), and weight of piglet at farrowing ($P = 0.003$) and 21 day postpartum ($P = 0.006$), but did not affect lactation feed intake of sows.

Maximizing voluntary feed intake of sows is the core of lactation feeding strategies, because depressed feed intake is thought to be a major contributor to greater magnitude of BW and backfat lost, which might adversely affect reproductive performance and lifetime productivity of sows (Koketsu et al., 1996; Eissen et al., 2003; Anil et al., 2006). Sulabo et al. (2010) reported that ad libitum-fed lactating sows had less BW loss ($P < 0.01$), improved

total and daily litter weight gain ($P < 0.04$), and a greater percentage of sows returning to estrus by day 14 after weaning ($P < 0.03$) when compared to restricted-fed sows. Koketsu et al. (1996) analyzed data records for 20,296 lactating sows on 30 commercial farms in the US and demonstrated that the sows with the rapid increase of feed intake during lactation had significantly shorter ($P < 0.01$) weaning-to-first service interval and weaning-to-conception interval than the sows that had lower feed intake. Furthermore, the feed intake pattern of lactation also suggested impacts on reproductive performance and longevity of sows. Sows with greater feed intake during the early to mid-lactation were demonstrated to have improved wean to estrus interval and reduced chance to be culled from the breeding herd; while higher feeding intake during mid- to late lactation could result in greater litter weight at weaning (Koketsu et al., 1996; Koketsu et al., 1997; Anil et al., 2006).

2.2.3.2 Protein

Adequate intake of protein from diets is critical for sows to maintain a high level of weaned pig output over the reproductive life (Boyd et al., 2000). The intensive genetic selection of breeding females has resulted in increased mature body size, reduced piglet mortality, and greater prolificacy of sows, and thus requires a higher demand of nutrients from the diet (Whittemore, 1996; O'Dowd et al., 1997). However, the consequence of such selection might also have led to decreased voluntary feed intake, which compromises protein and energy intake (Eissen et al., 2000). Severe and chronic mobilization of the body reserve of protein appears to have detrimental effects on wean to estrus interval and performance of subsequent reproductive cycles, and thus impacts lifetime productivity of sows (Boyd et al., 2000).

Previous studies showed that increased dietary protein level during gestation resulted in greater gestation BW gain (Holden et al., 1968; Baker et al., 1970; Shields et al., 1985; Heo et al., 2008). However, the results of lactation BW loss were conflicting. Baker et al. (1970) reported significant greater BW loss ($P < 0.05$) during lactation when CP levels in gestation diets increased from 8.7 to 16.0%; moreover, Holden et al. (1968) reported that lactation BW loss tended to increase as dietary CP levels elevated from 8 to 20% during gestation for four reproductive cycles. In contrast, Mahan (1998) reported no significant difference between 2 gestation diets containing CP of 13 and 16% ($P > 0.05$). Shields et al. (1985) indicated that sows fed 5% CP diet during pregnancy had more maternal fat, but less protein deposited when compared with sows fed a diet that contained 14% CP ($P < 0.05$), and those sows fed with 5% CP diet mobilized body protein reserve during the last trimester of pregnancy ($P < 0.01$). Pettigrew and Yang (1997) reported that a sufficient supply of protein and amino acids during gestation allowed sows to maintain productivity, and high body protein content of sows could maximize milk production and subsequent reproductive performance. Moreover, Greenhalgh et al. (1977) reported that sows fed a low protein diet during gestation (9% CP) and lactation (13% CP) exhibited longer weaning to estrus interval when compared with sows fed high protein diets. However, a moderate reduction of CP levels during gestation (16.0 to 11.3%) and increase during lactation (18.0 to 16.0%) has been demonstrated to reduce BW and backfat loss over 3 parities and shorten weaning-to-conception intervals, and eventually resulted in fewer sows failing to produce 3 litters and culled for reproductive failure (O'Dowd et al., 1997). The authors speculated the underlying rationale might be that, by moderately restricting the amount of essential amino

acids available for lean tissue growth, metabolizable energy must be diverted to build up body fat reserve for subsequent lactation and next reproductive cycles.

The larger litter size and reduced piglet mortality require modern sows to have high milk yield, but voluntary feed intake during lactation is often insufficient to satisfy the demands of nutrients for milk production (Dourmad, 1988; Eissen et al., 2000). As a result, body reserve of fat and protein must be mobilized, and excessive mobilization of body protein reserve during lactation may adversely affect litter growth and ovarian function (Clowes et al., 2003). Yang et al. (2000) demonstrated that increasing dietary CP levels from 14.67 to 28.82% during lactation linearly increased voluntary feed intake ($P < 0.05$), quadratically increased litter weight gain ($P < 0.05$), but did not influence sow weight change or weaning-to-estrus interval over 3 parities. Shields et al. (1985) reported a quadratic increase of sow feed intake and pig weight gain during lactation ($P < 0.05$) as CP levels of lactation diets increased from 5 to 23%, with a peak at 14%. Moreover, maternal protein and fat deposition also have been found to increase with increasing dietary CP levels ($P < 0.05$). However, declining dietary lysine levels (1.3 to 1.0%) during gestation has been reported to decrease the levels of total solids, protein, and solids-non-fat in colostrum and milk, and might result in poorer litter performance (Heo et al., 2008). It has been reported that protein restricted lactating sows had a lower percentage to return to estrus within 8 days after weaning, and a reduction in the ovulation rate when compared with sows that had no protein restriction during lactation; furthermore, the protein-restricted sows also were found to have more body protein loss and lower plasma concentration of insulin-like growth factor-1 (IGF-1) at the end of lactation period (Quesnel et al., 2005a; Quesnel et al., 2005b). Insulin-like growth factor-1 has anabolic effects on protein deposition in muscle

tissue; the reduction of plasma IGF-1 levels at weaning might suggest the lean tissue mobilization has been promoted. The age of sows might also have influenced the response to greater protein supply in diets, lactating multiparous sows fed high CP (or lysine) diets have been demonstrated to have greater milk production than the ones fed low CP (or lysine) diets (Revell et al., 1998; Kusina et al., 1999); however, increasing dietary lysine level for primiparous sows did not affect milk yield (Touchette et al., 1998).

2.2.3.3 Energy

Reproductive failure has been documented as the largest single cause for culling sows (Table 2.4). One of the principal causes of sow infertility is excessive loss of body reserves during lactation, which is normally because of inadequate lactation feed intake (Ren, 2016). Over recent years, genetically improved gilts have been introduced to the breeding herd, which not only are leaner and heavier at first mating than their counterparts of decades ago but have lower levels of body fat (Hughes et al., 2010). Such a situation leads to the consequences that it is hard to maintain their body condition and keep them in the breeding herds for a long breeding life (Hughes and Varley, 2003). Numerous studies have been conducted to investigate the influence of gilt body composition at first breeding on reproductive longevity, and some of them concluded that there were positive relationships between gilt backfat depth at first mating and the number of litters produced (Gueblez et al., 1985; Gaughan et al., 1995; Brisbane and Chenais, 1996). However, some other studies indicated that body condition of gilts at first breeding is not related to culling rate or lifetime productivity (Kirkwood et al., 1988; Young et al., 1990a; Newton and Mahan, 1993; Rozeboom et al., 1996). The discrepancy among the conclusions made in these studies might be due to the differences in experimental methods. Rather than focus on the backfat

depth at first breeding, Houde et al. (2010) investigated the effects of controlling the backfat depth at a fixed level during each breeding for 9 parities. The results indicated that the breeding herd with a maintained steady backfat depth on each time of breeding had a greater number of piglets born alive over 9 parities, compared with the other breeding herd with a marked decrease of backfat depth between parities 2 to 5. Therefore, one may infer from these results that maintaining backfat depth throughout the reproductive life might be more critical for lifetime productivity of sows than fixing this parameter at first breeding alone.

Thingnes et al. (2015b) reported that increasing daily energy intake by about 25% during gilt development (from BW of 25 kg to the first mating, 10.6 to 22.9 or 13.2 to 29.0 MJ of NE per day,) and mid-gestation of the first parity (d 42 to 94 of gestation, 22.3 or 27.3 MJ of NE per day) did not affect gilt and litter performance, but significantly reduced the probability of culling at the end of parity 1 ($P < 0.05$). With the same group of reproductive females, Thingnes et al. (2015a) continued monitoring the reproductive performance of sows up to 8 parities with common gestation and lactation diets. The overall results indicated that sows that received higher energy levels during gilt development and mid-gestation of the first parity showed lower risk of removal in early parities, had numerically the longest reproductive life-span in the breeding herd, as well as greater lifetime production of total born piglets ($P < 0.05$), born alive piglets ($P < 0.10$), and weaned piglets (numerically) per sow. Young et al. (1990b) also reported that sows that received low energy supply (5.31 Mcal of DE per day) had significant higher culling rate when compared with sows that received medium or high energy supply (6.98 or 8.65 Mcal of DE per day) during gestation over 4 parities, and the authors stated the major reason for the high culling

rate was failure to maintain backfat thickness throughout multiple reproductive cycles. Frobish et al. (1973) investigated the effects of increasing daily energy intake during gestation (3.0, 4.5, 6.0, and 7.5 Mcal ME daily) on long-term reproductive performance of sows (3 reproductive cycles), the results demonstrated that the number of sows that completed 3 reproductive cycles tended to be fewer for the highest level of energy supply due to leg abnormalities. However, other studies suggested the greater culling rate was found for sows with lower energy supply during gestation (Walker, 1983; Whittemore et al., 1988; Simmins et al., 1992). Results of those previous studies may suggest that neither inadequate nor excessive energy supply would benefit lifetime performance of sows.

Energy supply during gestation may affect fetal development. Liu et al. (2016b) reported that gilts fed a high-energy diet (control diet + 4.6% soybean oil) during gestation resulted in increased BW, small intestine weight, and villus height for fetuses at day 90 of gestation and piglets at birth and weaning, when compared to gilts fed control diet during gestation. Moreover, the authors also found the increased gene and protein expression of the IGF-1 receptor, as well as improved lactase and sucrase activity in the small intestine of fetuses from high-energy fed gilts. Cao et al. (2014) fed gilts with either over- (150% of NRC recommendations) or undernutrition diets (75% of NRC recommendations) during the entire gestation, and found significant increase in mRNA expression of sodium-dependent glucose transporter 1, glucose transporter 2, peptide transporter 1, and glucagon-like peptide-2 receptor in jejunum of newborn or weaned piglets from overnutrition group gilts. The increased expression of nutrient transporters and activity of digestive enzymes might suggest a better litter performance during lactation, and thus potentially reduce the chance to cull the breeding females because of poor performance.

Lactation is the period that most sows are under severe catabolic conditions due to the massive production of milk, which is in excess of limited nutrient intake (Kim and Easter, 2003). An extended catabolic condition during lactation can lead to greater oxidative stress and compromise sow longevity and lifetime productivity (Kim et al., 2013). When voluntary feed intake limits adequate nutrient supply, providing a diet with greater nutrient levels and higher digestibility can be beneficial. Smits et al. (2013) increased dietary DE levels from 3.11 to 3.66 Mcal per kg for primiparous sows during a 27-d lactation period, and then all experimental sows were fed standard diets throughout the second reproductive cycle. The results demonstrated that reduced BW loss as dietary energy level increased (linear, $P < 0.001$) during the first parity, and the proportion of sows staying the herd until the end of second parity was maximized when sows were fed diets containing at least 3.39 Mcal of DE per kg of diet ($P < 0.05$). Furthermore, increasing energy supply during gestation and lactation or lactation alone has been reported to minimize weaning-to-estrus interval and increase the percentage of sows back to estrus within 7, 14, and 21 day after weaning (Reese et al., 1982; Nelssen et al., 1985; Coffey et al., 1994a).

2.2.3.4 Minerals

The length of time that reproductive females stay in the breeding herd may be influenced by the extent of tissue mineral storage and their genetic productivity potential (Mahan, 1990). The prolific modern sows are producing larger and heavier litters than their counterparts decades ago, which requires greater minerals to be mobilized from body storage for every single reproductive cycle. Mahan and Newton (1995) concluded that the tissue contents of Ca, P, Mg, Zn, and Cu were lower ($P < 0.05$) in sows that completed 3 reproductive cycles than in the similarly aged, nongravid gilts. The depleted body reserve

of minerals might depress reproductive performance of sows, and eventually result in sow removal from the breeding herds.

Macro-minerals

Calcium and phosphorous.

Dietary requirements for calcium (Ca) and phosphorus (P) increase dramatically during late gestation and lactation when demands for fetal development and milk secretion are highest (McDowell, 2003a). Previous studies showed that increased dietary Ca and P levels by 50% on the basis of NRC recommendations during gilt development did not affect litter size or litter gain of subsequent parities (Nimmo et al., 1981b; Kornegay et al., 1985). Similarly, providing higher dietary Ca and P levels (40 to 50% higher than control diets) during gestation and lactation did not affect litter performance either (Kornegay et al., 1973; Harmon et al., 1975; Mahan and Fetter, 1982). However, dietary Ca and P levels may influence sow performance and bone integrity, thus affect the longevity of sows. Everts et al. (1998a) fed either low or high Ca and P diets (0.81 and 0.65% vs. 0.94 and 0.72% of Ca and total P, respectively) to gilts during lactation for 3 parities; the results showed that Ca and P exhibited negative balance for both dietary treatments, and the low Ca and P diet had poorer Ca retention during parity 2 and 3 ($P < 0.005$), and tended to have a poorer P retention during parity 2 ($P = 0.056$). Maxson and Mahan (1986) reported that nongravid females had higher percentage of bone ash, bending moment, and shaft thickness, when compared with same-aged reproducing sows after 2 reproductive cycles, even though reproducing sows consumed lactation diets with higher Ca and P levels (1.17 and 0.90% vs. 0.80 and 0.60% of Ca and total P, respectively). Furthermore, Kornegay et al. (1973) reported that the number of sows completing 5 reproductive cycles was fewer for the ones

that received low Ca and P diet (10.3 and 11.0 g of Ca and total P, respectively, per day) as compared to the ones that received the high Ca and P diet (15.5 and 15.0 g of Ca and total P, respectively, per day). And Nimmo et al. (1981a) also reported that 30% of sows fed diets low in both Ca (0.65%) and total P (0.50%) during growth and gestation were subsequently removed for locomotive problems, compared to 0% of sows fed control diets (0.975% of Ca and 0.75% of total P).

Lameness is one of the major reasons for sow culling in the US swine herds, which accounted for 15.2% of sows removed from the breeding herd (Anil et al., 2009). Both Ca and P are essential elements in soft tissue metabolism, but their involvement in maintaining bone integrity and in preventing lameness remains controversial (Mahan, 1990). Nimmo et al. (1981a) reported that higher dietary Ca and P levels (150% of control diet) during gilt development increased average peak force and bending moment of the third and fourth metatarsal bones ($P < 0.001$) at the end of the growth phase; and higher dietary Ca and P levels (150% of control diet) during gestation also increased peak force of the fourth metatarsal bone ($P < 0.05$) at the end of lactation. However, other studies showed that increased dietary Ca and P levels by 50% during gilt development did not affect incidence and severity of lesions on the toes, overall structural soundness, or longevity of sows kept for 3 parities (Arhur et al., 1983; Arthur et al., 1983).

Magnesium

Magnesium (Mg) is the third most abundant mineral constituent of bone, following Ca and P. It plays significant roles in more than 300 different enzyme systems and thus participates in a huge array of biological reactions (Patience and Zijlstra, 2001). The current edition of the NRC suggests that gestation and lactating sows require 0.06% of Mg in diets

(NRC, 2012), which is much lower than the Mg content from the major feed ingredients (0.20 to 0.25 %) in typical corn and soybean meal (SBM) breeding herd diets. Everts et al. (1998a, 1998b) fed sows with diets that contained at least 0.21% of Mg throughout gestation and lactation for 3 parities, and the results showed that Mg retention stayed positive for both gestation and lactation for each parity. Some recent studies have shown that adding Mg (in the form of $MgSO_4$) up to 0.045 or 0.060% into basal gestation and lactation diets resulted in decline of weaning-to-estrus interval (linear or quadratic, $P < 0.05$), as well as increase of fecal moisture content and decrease of constipation rate (linear, $P < 0.05$) of sows (Hou et al., 2014; Zang et al., 2014).

Micro-minerals

Chromium

Chromium (Cr) is an essential trace mineral that is pivotal in glucose, protein and fat metabolism in animal tissue (Ohh and Lee, 2005). Although it is not listed in the NRC (2012) requirement estimates for gestating and lactating sows, the uniform response of increased prolificacy to increased dietary Cr has been reported in many studies (Lindemann, 2007). Lindemann et al. (1995) first reported that diets supplemented with Cr (200 vs. 0 μg/kg) from chromium picolinate (CrPic) increased the number of live pigs born in sows through 2 parities when compared to a diet without Cr supplementation. The effects of CrPic on reproductive females have been confirmed in recent studies in both research and commercial settings (Hagen et al., 2000; Lindemann et al., 2004; Real et al., 2008; Wang et al., 2013). Furthermore, Lindemann et al. (1995) also reported that pre- and post feeding insulin to glucose ratio significantly decreased ($P < 0.003$) for sows fed high Cr diets than the low Cr diet, which indicated a greater efficiency of insulin action in mid-gestation.

These results are in agreement with Wang et al. (2013), who reported that the concentrations of Cr, insulin, glucose, and serum urea nitrogen in serum decreased significantly ($P < 0.05$) on day 70 and 110 of gestation with 400 μg/kg of dietary Cr supplementation. Chromium-methionine (CrMet) is another form of organic Cr that has been evaluated in reproducing females. It was reported that up to 400 μg/kg of Cr from CrMet in gestation and lactation diets increased sow feed intake and decreased BW loss during lactation ($P < 0.10$), increased number of piglets born alive ($P < 0.01$), and diminished weaning-to-estrus interval ($P < 0.01$) of multiparous sows (Perez-Mendoza et al., 2003; Pérez-Mendoza et al., 2003; Romo et al., 2005).

Copper

Copper is involved in specific Cu-dependent enzymes that regulate hemoglobin synthesis, skeleton, collagen and myelin formation (Hill and Spears, 2001). Ma (2011) reported that Cu contents in the fetus and fetal liver increased (quadratic and cubic, $P < 0.01$) from day 43 to 108 of gestation for first-parity gilts. Because sows are depleting the body reserves of Cu as reproductive cycles advanced (Mahan and Newton, 1995), one may speculate that body reserve of Cu may become less available to transfer to fetuses for older sows, which may affect sow and litter performance. The current NRC requirement estimates (NRC, 2012) of Cu during gestation and lactation are 10 and 20 mg/kg. Cao and Chavez (1995a) reported that sows fed low Cu diets (2.13 mg/kg) during late gestation and lactation had smaller litter size and lower Cu concentrations in colostrum and milk than sows fed high Cu diets (12.25 mg/kg). In contrast, Cromwell et al. (1993) has demonstrated that feeding sows with diets containing 250 mg/kg of Cu from copper sulfate ($CuSO_4$) for up to 6 reproductive cycles decreased culling rate ($P < 0.01$) and weaning-to-estrus interval

($P < 0.10$), and improved piglet weight at birth ($P < 0.001$) and weaning ($P < 0.01$), when compared with sows fed diets with no extra Cu added. Lillie and Frobish (1978) reported adding extra Cu as $CuSO_4$ at levels from 0 to 60 mg/kg in sow diets throughout 4 parities linearly increased the total and live adjusted birth weights; and Thacker (1991) suggested that top-dressing of 250 mg/kg of Cu ($CuSO_4$) during late gestation and lactation reduced piglet mortality, but did not affect sow or litter performance. In addition, Cu also participates in bone remodeling and cartilage formation, which is related to the feet and leg health of sows (van Riet et al., 2013). Copper deficiency may lead to a marked reduction in osteoblast activity and failure of bone deposition on the calcified cartilage matrix, as well as reduced joint rigidity, excessively flexed hocks, and crooked forelegs (McDowell, 2003b).

Iron

The fast-growing nursing pigs have substantial daily requirement of Fe, and the low concentration of Fe in milk results in the use of hepatic reserves of Fe (Mahan, 1990; Hill and Spears, 2001). Thus, prolonged hepatic Fe mobilization without an exogenous supply of Fe could eventually lead to anemia. In contrast, the adult female pig appears resistant to Fe deficiency due to the conservation of body Fe reservoirs stored in body tissue (Mahan, 1990). Ma (2011) has demonstrated that concentrations and contents of maternal liver Fe were not changed from day 43 to 108 of gestation; while fetal liver Fe content increased significantly (quadratic, $P < 0.01$). A single dose of 200 mg of Fe dextran has been commonly administrated shortly after birth to address the physiological iron deficiency of piglets in practice (NRC, 2012). Meanwhile, efforts are still being made to improve Fe status and performance of nursing piglets via improving Fe status for sows. Guise and

Penny (1990) reported that intramuscular injection of 1600 mg Fe (as gleptoferron) for gestating sows at 3 weeks before expected farrowing dates resulted in the numerical improvement in the number of piglets born alive and litter weight gain, and suggested an overall benefit of approximately 0.45 pigs per sow per year. Higher dietary Fe levels (256 vs. 114 mg/kg) during gestation and lactation for multiparous sows showed a greater daily Fe retention, higher placental Fe content at the end of pregnancy, and improved litter size and weight (Buffler et al., 2017). Moreover, supplying organic Fe complex during late gestation and lactation resulted in increased total Fe binding capacity (P = 0.08) and ceruloplasmin (Cp) activity (P = 0.05) on day 10 of lactation, as well as improved concentrations of hemoglobin (P = 0.08) and serum Fe (P = 0.081) on day 21 of lactation for nursing piglets, when compared with Fe sulfate (Wang et al., 2014).

Manganese

Manganese is critical for growth, fertility, and bone organic matrix formation of animals (Hill and Spears, 2001). Because Mn is found abundantly in grain diets, it is not thought to be deficient in a typical corn-SBM diet for swine (Hill, 2013). Manganese-deficient diets have been demonstrated to cause irregular or complete absence of estrus and reduced pig birth weights in sows, as well as increased fat deposition and skeletal abnormalities in developing gilts (Plumlee et al., 1956; Christianson, 1990). In addition, partially substituted dietary inorganic Mn, Zn, and Cu with their organic counterparts for 1 or 2 complete reproductive cycles has resulted in significantly lower odds of the higher versus the lower hoof lesion score, lower incidence of histologic changes on the claws, and higher density as well as smaller vertical and horizontal diameters of the horn tubules (Lisgara et al., 2016; Varagka et al., 2016).

Selenium

It is well known that pregnancy is a state of oxidative stress due to increased placental mitochondrial activity and production of reactive oxygen species (ROS) (Burton and Jauniaux, 2011). Although ROS serve as key signaling molecules in physiological processes in the female reproductive tract, excessive ROS may result in oxidative stress that impairs milk production, reproductive performance, and finally longevity of sows (Zhao, 2011; Rizzo et al., 2012). The sow mortality rate has risen during the past 15 years from 6% to almost 10% in the US (Figure 2.1), which might suggest an associated increasing systemic oxidative stress throughout late gestation and lactation (Berchieri-Ronchi et al., 2010). Mahan (2000) reported that serum selenium (Se) and glutathione peroxidase (GSH-Px) activity showed a decline during the latter part of gestation and lactation, which might indicate that Se status of reproductive sows needs to be reevaluated.

Inorganic Se forms, including selenite and selenate, have been used for the last 50 years in swine diets (Surai and Fisinin, 2016). However, the limitations of using inorganic Se are known as: high toxicity, interaction with other minerals, low efficiency of transfer to milk, meat, and eggs, inability to build and maintain Se reserve in the body, as well as prooxidant effect (Surai, 2006; Surai and Fisinin, 2014, 2016). Mahan and Peters (2004) fed gilts with diets containing increasing levels of Se (0, 0.15, and 0.30 mg/kg) in the form of either sodium selenite or Se yeast from 27.6 kg BW (after the nursery phase) until the end of the fourth parity, and concluded that serum Se and GSH-Px activity decreased from day 70 to 110 of gestation in all treatments, but increased at weaning ($P < 0.01$) in the Se-fortified groups regardless of Se sources; increasing dietary Se levels also increased tissue Se contents at birth ($P < 0.01$), as well as increased Se concentrations in milk and colostrum

($P < 0.01$) for both Se sources. However, compared with sows fed inorganic Se diets, the organic Se group had greater Se concentration in sow tissue ($P < 0.01$) and piglet tissue at birth ($P < 0.01$) by the end of fourth parity; and Se concentration in colostrum and milk were greater for organic Se fed sows than inorganic Se fed sows during the overall 4 parities ($P < 0.01$). These results are in agreement with other studies, which show that supplemental Se (0.3 mg/kg) from Se yeast throughout gestation and lactation improved the antioxidant capacity for sow and piglets at birth and weaning, as well as increased Se concentrations in blood, milk, colostrum, and piglet tissues (Mahan, 2000; Yoon and McMillan, 2006; Quesnel et al., 2008; Svoboda et al., 2008; Chen et al., 2016b, a). Selenomethionine is another source of organic Se for swine diets, 2 recent studies reported that supplementation of 0.3 mg/kg of Se from selenomethionine in sow diets during late-gestation and lactation elevated Se concentrations in milk, colostrum, and various piglet tissues ($P < 0.05$), improved antioxidant status of sows and piglets, and increased pancreatic amylase, trypsin, and lipase activity ($P < 0.05$) of piglets at weaning (Hu et al., 2011; Zhan et al., 2011).

Zinc

The current NRC estimates that the dietary requirement of Zn is 100 mg/kg (NRC, 2012). Inadequate intake of Zn during gestation and lactation may cause reproductive failure (Smith and Akinbamijo, 2000), and thus impair sow longevity. Wegger and Palludan (1978) reported that Zn deficiency during the last trimester of pregnancy resulted in delayed farrowing. Moreover, some other studies demonstrated that low dietary Zn levels (13 and 33 mg/kg) during gestation and lactation decreased concentrations of Zn in plasma of sows, as well as in liver and femur of piglets. However, low Zn levels in gestation and lactation diets did not affect sow and litter performance (Hedges et al., 1976; Kalinowski and Chavez,

1984). Hill et al. (1983b) fed gilts with diets containing 0, 50, 500, and 5,000 mg/kg of Zn (ZnO) from 30 kg BW until the end of the second parity, and demonstrated that the lowest Zn group had a higher number of abnormal pigs per litter, whereas the highest Zn group had fewer and lighter weaned pigs, when compared to intermediate Zn groups. In addition, the results suggested that higher dietary Zn level might depress deposition of Cu and Fe into various organs, and might induce higher incidence of osteochondrosis in humeral-radioulnar joints of sows.

The commonly used Zn sources in practice are ZnO and $ZnSO_4$ (NRC, 2012). However, organic forms of Zn also have been evaluated due to greater relative bioavailability. It was reported that sows that received a diet containing 200 mg/kg Zn from mixed sources [100 mg/kg from each of ZnAA (Zn AA complex) and $ZnSO_4$] had increased litter birth weight ($P < 0.10$) compared to sows that consumed pure $ZnSO_4$ diet (Payne et al., 2006). However, van Riet et al. (2016) reported that Zn sources (ZnAA or ZnO) did not affect levels of plasma Zn or serum metallothionein, or apparent Zn absorption of late gestation sows (d 86 to 106 of gestation).

2.2.3.5 Vitamins

There are a total of 13 vitamins listed in NRC requirement (4 fat-soluble and 9 water-soluble vitamins), and most of them have been found to influence reproductive performance of sows (Pettigrew and Tokach, 1991; NRC, 2012). In contrast to substantially improved prolificacy of reproducing females during the last 10 to 20 years, dietary requirement estimates for 10 out of these 13 vitamins have not been changed since NRC (1988) (only vitamin A, D, and folacin have increased requirement estimates). A recent survey from 18 US swine production systems representing approximately 2.3 million sows

indicated that average inclusion rate of vitamins during gestation and lactation were 1.3 to 7.3 times of NRC (2012) recommendations, except for choline (Flohr et al., 2016a). The greater inclusion rates might be partially explained as adding a safety margin to compensate for the vitamin loss from feed processing and storage, but they may still suggest that prolific modern sows might need more vitamins to maximize reproductive performance and longevity.

Fat-soluble vitamins

Vitamin A

Vitamin A is essential for reproduction because it is required for ovarian follicle maturation, maintaining proper functioning of corpora lutea and epithelial cells of the oviduct as well as uterine and cervix environment, and facilitating embryonic development (Matte and Lauridsen, 2013). Vitamin A deficiency in sows may result in the resorption of the fetus, abortion, or birth of dead offspring (McDowell, 2000c). Brief and Chew (1985) examined the effects of providing vitamin A or β-carotene via the diet or injection throughout gestation and lactation on reproductive performance of primiparous sows that were depleted of vitamin A and concluded that gilts injected with either β-carotene or vitamin A had more piglets per litter at birth and weaning. Moreover, Lindemann et al. (2008) injected increasing dosages of vitamin A (0, 250,000, and 500,000 IU) to mixed-parity sows (parity 1 to 6) at weaning and breeding days, and then provided vitamin A sufficient diets throughout gestation and lactation. The results demonstrated improvements on numbers of piglets born total, born alive, and weaned alive in parity 1 and 2 sows, but not in sows of parity 3 to 6. Nevertheless, the effects of vitamin A administration on litter size is conflicting. Coffey and Britt (1993) reported that one injection of increasing dosage

(0, 50, 100, and 200 mg) of ß-carotene on the day of weaning increased subsequent litter size at birth in multiparous sows, but not in primiparous sows. Pusateri et al. (1999) indicated that a single injection of vitamin A (1,000,000 IU) at any time from weaning to subsequent farrowing did not increase litter size in sows fed with vitamin A adequate diet.

Vitamin D

Vitamin D is historically recognized for its relevance to bone health and calcium homeostasis. The biological actions of vitamin D are exerted through vitamin D receptor, a soluble protein that is distributed in various tissues, including the reproductive system. This may suggest that vitamin D is involved in female reproduction (Shahrokhi et al., 2016).

Goff et al. (1984) found that intramuscular injection of 5,000,000 IU of cholecalciferol (vitamin D_3) 20 d prepartum resulted in a high degree of correlation ($r > 0.73$) between sow and piglet plasma concentrations of 25-hydroxycholecalciferol (25-OH-D_3), 24,25-dihydroxycholecalciferol (24,25-$(OH)_2$-D_3), and 25,26-dihydroxycholecalciferol (25,26-$(OH)_2$-D_3), which indicated that vitamin D status of piglets could be improved by elevating sow vitamin D status. In addition, feeding sows with diets containing increasing levels of vitamin D_3 for short-term (d 35 to 65 of gestation) or long-term (throughout entire gestation and lactation) significantly increased sow serum 25-OH-D_3 on day 100 of gestation, at farrowing, and at weaning, increased milk vitamin D_3 levels, as well as increased piglet serum 25-OH-D_3 at birth and weaning (Flohr et al., 2014; Flohr et al., 2016b).

Cholecalciferol is the most common source of supplemental vitamin D in swine diets. In recent years, 25-OH-D_3 has become commercially available and has been evaluated in sow diets. Lauridsen et al. (2010) fed gilts with diets containing 200, 800, 1,400, and 2,000 IU/kg of vitamin D from either cholecalciferol or 25-OH-D_3 from the first day of mating

until weaning, and reported that concentrations of 25-OH-D_3 were greater in the plasma of sows receiving 25-OH-D_3 than in sows receiving vitamin D_3 during lactation except for the lowest dietary dose (200 IU/kg). Weber et al. (2014a) conducted a 4-parity study to investigate the effects of different source of vitamin D (vitamin D_3 and 25-OH-D_3) on reproductive performance; and results have demonstrated that sows fed 25-OH-D_3 diet had greater plasma 25-OH-D_3 on breeding day, 28 and 80 days post-insemination, and 5 and 28 days postpartum, as well as had a greater 25-OH-D_3 content in milk when compared with the vitamin D_3 diet.

The active form of vitamin D (1,25-$(OH)_2$-D_3) maintains plasma Ca and P levels for mineralization of unmineralized bone matrix (McDowell, 2000d). Impaired availability of vitamin D will decrease the availability of Ca and P in blood, which may have adverse consequences for bones (van Riet et al., 2013). Large quantities of Ca and P are mobilized from the skeleton to meet increasing requirements for fetal growth and milk production during gestation and lactation (Halloran et al., 1979). Therefore, sufficient plasma vitamin D may benefit feet and leg health, and decrease the risk of culling for locomotion problems.

Vitamin E

Vitamin E concentrations in maternal plasma normally decline from late gestation to parturition, reaching a nadir around farrowing and returning towards baseline levels within a few weeks of lactation (Hidiroglou et al., 1993). During the same physiological stage, lymphocyte DNA damage, which is used to determine oxidative stress status, has shown a significant elevation from mid-gestation until lactation compared to early gestation (Berchieri-Ronchi et al., 2010). Since vitamin E is one of the primary antioxidants in the body's oxygen-generated defense system (Mahan, 2001), these results might suggest that

the increased oxidative stress during late gestation and lactation could be associated with depleted vitamin E status of sows.

Mahan (1994) investigated increasing levels of the commercially-used source of vitamin E, DL-α-tocopheryl acetate (22, 44, or 66 IU/kg diet), over a 5-parity period on reproductive performance and on α-tocopherol concentration in serum, colostrum, and milk of the sows. Increasing dietary vitamin E improved number of piglet born total (linear, $P < 0.01$) and alive (quadratic, $P < 0.10$), increased serum α-tocopherol levels in serum, colostrum, and milk (linear, $P < 0.01$), as well as decreased the incidence of mastitis, metritis, and agalactia (quadratic, $P < 0.05$). Furthermore, increased dietary vitamin E level has been demonstrated to increase total antioxidant capacity in piglet serum and liver at birth and weaning, but failed to improve antioxidant status of sows (Chen et al., 2016b; Wang et al., 2017).

Synthetic vitamin E (DL-α-tocopherol; all rac-α-tocopherol) is a combination of the 8 stereoisomers, whereas natural vitamin E (D-α-tocopherol; RRR-α-tocopherol) comprises only the RRR stereoisomer. Relative bioavailability of the 2 vitamin E sources has been evaluated for sows. Shelton et al. (2014) reported that the bioavailability coefficients for D-α-tocopherol relative to DL-α-tocopherol ranged from 1.9 to 4.2 for the sow and pig plasma α-tocopherol, 2.9 to 3.6 for colostrum α-tocopherol, 1.6 for milk α-tocopherol, and 1.7 to 2.0 for pig heart and liver α-tocopherol. These results are in agreement with Mahan et al. (2000), who has demonstrated that D-α-tocopherol has a higher equivalency than DL-α-tocopherol on an IU basis.

Water-soluble vitamins

Biotin

Biotin is present in most common feedstuffs in, what is thought to be, more-than-adequate amounts, and its bioavailability in corn and SBM is considered as 100% for animals (McDowell, 2000a; NRC, 2012). The effects of supplementation of biotin (150 to 440 µg/kg) have been reported to improve conception rate at first estrus postpartum, reduce weaning-to-estrus interval, and more pigs born alive (Brooks et al., 1977; Bryant et al., 1985). However, some other studies (440 to 500 µg/kg) failed to show a significant improvement on reproductive performance from supplemental biotin (Greer et al., 1991; Lewis et al., 1991; Watkins et al., 1991). Furthermore, Brooks et al. (1977) showed that supplementation of 150 and 250 µg/kg of biotin in gestation and lactation diets for 6 months decreased the number of foot lesions by 28%. Penny et al. (1980) reported that providing 1,160 and 2,320 µg of biotin per day during gestation and lactation for 12 months, respectively, significantly reduced lesions of heel erosion, and decreased the severity and number of lesions of the lateral hind claws. Therefore, biotin supplementation of sow diets might be beneficial to sow longevity by reducing hoof lesions.

Folic acid

Folic acid is essential for all tissues with a high rate of cellular division and growth because it is involved in the transfer and utilization of monocarbon units for metabolic processes related to protein and DNA synthesis (Matte et al., 2006). It was reported that plasma concentrations of folates (N-5-methyltetrahydrofolic acid) (Matte et al., 1984b; Thaler et al., 1989) and high-affinity plasma folate binders (O'Connor and Picciano, 1993) decreased during gestation of both gilts and sows. Moreover, these authors further stated

that the major drops of plasma folates happened between weaning and mating, and day 30 to 60 of gestation, but plasma folate levels tended to increase from day 60 to 110 of gestation. It has been reported that 10 intramuscular injections of 15 mg folic acid during early and mid-gestation restored plasma folates level on day 60 of gestation when compared sows without folic acid injection (Matte et al., 1984a). Moreover, dietary supplementation with 5 mg/kg of folic acid from weaning to day 30 of gestation significantly improved serum folate concentrations ($P < 0.001$) of sows on day 30 postmating (Tremblay et al., 1989). Supplemental folic acid during gestation has been associated with 7 to 12% improvement in the number of piglets born alive regardless of the methods of administration (through injection or in diets) (Matte et al., 1984a; Thaler et al., 1989; Matte et al., 1992; Lindemann, 1993). Harper et al. (1996) fed sows with diets containing 2 mg/kg of folic acid from 21 days prior to breeding until day 42 to 48 of gestation, and reported that although the number of live fetuses and fetal survival at day 45 of gestation was not affected by folic acid supplementation; fetal pig weight, length, protein, and RNA content were increased ($P < 0.05$) with folic acid treatment, which suggested enhanced development of embryo or fetal tissues. Matte et al. (1996) also reported improved embryonic survival in sows fed folic acid supplemented diet (15 mg/kg) from 2 weeks before mating and day 12 or 15 postmating, and the improvements might be linked to changes in the secretion of uterine prostaglandins and possibly embryonic development.

Vitamin B_{12}

Vitamin B_{12}, or cyanocobalamin, as a coenzyme is involved in the de novo synthesis of labile methyl groups and their transfer to homocysteine to generate methionine (NRC, 2012). Deficiency of vitamin B_{12} may induce a local or systemic accumulation of

homocysteine, which is a strong pro-oxidant known to impair embryo development (Matte et al., 2006). Guay et al. (2002b) reported that plasma vitamin B_{12} was approximately 2-fold lower in gilts than in multiparous sows, and the lower vitamin B_{12} status in gilts might be linked to the fact that the demands for vitamin B_{12} are in competition between growth and maintenance of the gilt as well as development of fetuses; while multiparous sows are already matured, and thus have more vitamin B_{12} for reproductive functions (Matte et al., 2006). Also, Guay et al. (2002a) reported that vitamin B_{12} content in uterine secretions represented between 180 and 300% of the total content in plasma at day 15 of gestation, which suggests that vitamin B_{12} is required in significant amounts particularly during early gestation. A recent study showed that increasing dietary vitamin B_{12} levels during gestation (0, 20, 100, 200, and 400 μg/kg) increased overall vitamin B_{12} concentrations during gestation (linear and quadratic, $P < 0.01$) and at day 21 of lactation (linear and quadratic, $P < 0.04$), and decreased plasma overall homocysteine concentrations during gestation (linear, quadratic, and cubic, $P < 0.01$). Moreover, the broken-line analysis estimated that the concentrations of dietary vitamin B_{12} to maximize plasma vitamin B_{12} and minimize plasma homocysteine of sows during gestation were 164 and 93 μg/kg, respectively; which is greater than the vitamin B_{12} requirement estimated by the current NRC (15 μg/kg).

Niacin, pantothenic acid, and riboflavin

There are quite limited numbers of studies investigating the impacts of dietary niacin, pantothenic acid, and riboflavin on reproductive performance of sows during the past 30 years.

Niacin is unlikely to be deficient in adult pigs when sufficient tryptophan is present in diets because excess tryptophan can be metabolically converted to niacin (Firth and

Johnson, 1956). Ivers et al. (1993) concluded that supplementation of 33 mg/kg of niacin to gestating and lactating diets containing a low concentration (0.12%) of tryptophan did not affect reproductive performance of sows. Recently, Mosnier et al. (2009) reported that when dietary niacin level was above its requirement (45 vs. 10 mg/kg), plasma niacin levels of sows decreased during the first week of lactation, but then increased throughout the rest of lactation. It was suggested that niacin might have been transiently suboptimal in early lactation.

Riboflavin is indispensable for normal reproductive function. Esch et al. (1981) reported that cycling gilts became anestrous after receiving a riboflavin-deficient diet (0.77 mg/kg) for 63 d, while the gilts on a riboflavin adequate diet (4.07 mg/kg) exhibited normal estrus. Bazer and Zavy (1988) reported that oral administration of 100 mg riboflavin per day during day 4 to 10 of pregnancy increased conception rate, embryo survival, and litter size of gilts.

Pantothenic acid is important in sow fertility; insufficient dietary pantothenic acid could result in complete reproductive failure (McDowell, 2000b). Ullrey et al. (1954) reported that providing a diet containing 0.2 mg/kg of pantothenic acid to gilts for one month before breeding resulted in no pigs born at farrowing, atrophic and inactive ovaries, and immature reproductive system, when compared to sows that received diets containing 2.5 or 3.9 mg/kg of pantothenic acid. Teague et al. (1971) reported that providing 8.7 mg of pantothenic acid per day during gestation for 2 generations (4 farrowing) resulted in the appearance of well-defined deficiency symptoms in piglets farrowed by second-generation gilts and sows. These piglets showed locomotor symptoms including leg stiffness, no use of hind legs, or sitting on haunches at birth, and more frequently at 2 to 4 days post-

farrowing. Neither pantothenic acid supplementation in sow diet from mid-gestation nor early post-farrowing pantothenic acid injection in piglets prevented or alleviated the appearance of deficiency symptoms.

2.3 Copper metabolism in animals

2.3.1 Copper absorption and its regulation

The proximal small intestine is believed to be the primary site of Cu absorption for most animal species (McDowell, 2003b). However, a substantial amount of Cu can be absorbed from other parts through gastrointestinal tract as well. Van Campen and Mitchell (1965) reported 11.4% of ^{64}Cu was absorbed in the stomach of rats by placing radioisotope in the stomach (ligated at the pylorus) for 2 hours. Moreover, appreciable absorption of ^{64}Cu occurred from the proventriculus and stomach of chicks and mice, respectively (Starcher, 1969; Van Barneveld and Van Der Hamer, 1984).

Copper appears to be taken up by the intestinal epithelium by two mechanisms; saturable transport when luminal Cu concentration is low, and simple diffusion when Cu concentration is high (Linder, 1991). The former one needs some proteins localized on the apical membrane to facilitate the transport, while the latter one does not. Many factors influence the absorption of Cu, including the chemical form of Cu, dietary Cu levels, and physiological stage of animals. Generally speaking, absorption is greater in young and pregnant than mature animals, and in Cu-deficient than Cu-sufficient animals (McDowell, 2003b; Hill, 2013).

Biologically, Cu is essential for all organisms because it is involved in numerous metabolic processes. However, Cu exerts high redox activity, and excess Cu can generate damaging free radicals and be potentially toxic. With the purpose of keeping a balance

between acquiring sufficient Cu for cellular requirements and avoiding accumulation to levels that could lead to toxicity, animals have evolved an elaborate network of strategies to maintain Cu homeostasis (La Fontaine and Mercer, 2007). With regard to pig production, Cu concentration in sow colostrum and milk are relatively low, typically around 1.8 and 0.9 mg/L (Farmer, 2015); but pigs will be provided with diets that contain 150 mg/kg of Cu immediately after weaning until around 23 kg of BW, and then 80 to 50 mg/kg of Cu in diets until slaughter (Flohr et al., 2016a). These facts may indicate that pigs are capable of handling dramatic changes in the amount of Cu input throughout their entire life while maintaining Cu homeostasis.

Models of Cu homeostasis regulation in the enterocyte have been proposed (La Fontaine and Mercer, 2007; Lonnerdal, 2008). Figure 2.2 shows how Cu traffics among intracellular proteins in enterocyte under normal or high Cu exposure. Copper transporter 1 (Ctr1) is believed to be the primary protein responsible for importing dietary Cu across the brush border microvilli; in addition, divalent metal transporter 1 (Dmt1) is postulated to facilitate copper absorption in the intestine (Prohaska, 2008). Because Cu is absorbed as Cu^{+}, dietary Cu, which is likely in the form of Cu^{2+}, needs to be reduced before uptake at the apical membrane of the enterocyte (Lonnerdal, 2008). A family of metalloreductases, called Steap (six transmembrane epithelial antigen of the prostate) proteins, were shown to be expressed in the brush border membrane of duodenum epithelium with the function of reducing Cu^{2+} to Cu^{+} (Ohgami et al., 2006). Under conditions of normal environmental Cu concentrations, both Ctr1 and Dmt1 facilitate transportation of Cu^{+} from the intestinal lumen. However, when exposed to high Cu concentrations, Ctr1 has been reported to be rapidly endocytosed and degraded with the purpose of mediating Cu uptake and maintaining intracellular Cu

homeostasis (Petris et al., 2003). It has been reported that dietary supplementation of 225 mg/kg of Cu as either tribasic copper chloride (TBCC) or $CuSO_4$ resulted in numerically decreased mRNA expression of Ctr1 in the duodenum in weanling pigs (Huang et al., 2015).

After uptake by the enterocytes, Cu is most likely delivered to antioxidant 1 (Atox1), which is a Cu chaperone protein that delivers intracellular Cu to Cu transporting alpha-polypeptide ATPase (Atp7a) and then further delivers it to the *trans*-Golgi network (TGN) (Lutsenko et al., 2007). Moreover, some other Cu chaperone proteins, such as cytochrome c oxidase assembly protein 17 (Cox17) and Cu chaperone for Cu/Zn superoxide dismutase (Ccs), deliver intracellular Cu to mitochondria for incorporation into cytochrome c oxidase and to Cu/Zn superoxide dismutase (SOD), respectively. Metallothionein (MT) is an intracellular metal binding protein that binds Cu and acts as a detoxifier when Cu is in excess (Davis and Cousins, 2000). It has been reported that MT mRNA expression in the small intestine of rat pups increased at the higher Cu exposure (Bauerly et al., 2004), and that rats fed high amounts of Cu were found to have high MT concentrations in villous epithelial cells (Mullins and Fuentealba, 1998). These results may suggest that MT plays a protective role by limiting Cu transport across the basolateral membrane.

The export of Cu from the epithelial cell is mediated by Atp7a and Atp7b, which transport Cu across cellular membranes through a catalytic cycle with energy derived from ATP hydrolysis. The expression of Atp7a is found in the majority of tissues except for the liver, while Atp7b is expressed primarily in the liver, but also in kidney, intestine, and placenta (La Fontaine and Mercer, 2007). At normal conditions, both proteins reside at the TGN of the cell, where Cu is transported for incorporation into the Cu-dependent enzymes.

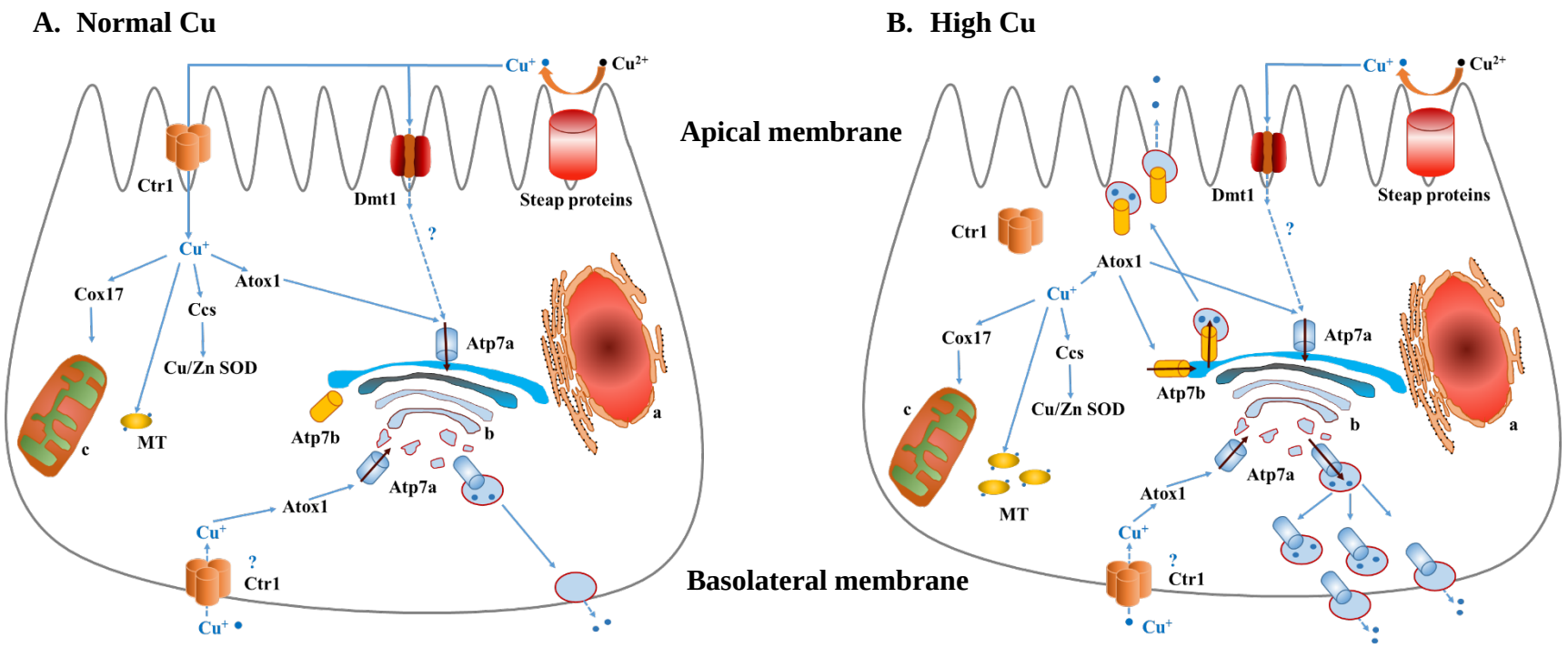

Figure 2.2. Proposed model of copper trafficking in the enterocyte under (A) normal Cu exposure and (B) high Cu exposure. a: nucleus and endoplasmic reticulum, b: trans-golgi network, c: mitochondria, Ctr1: copper transporter 1, Dmt1: divalent metal transporter 1, Cox17: cytochrome c oxidase assembly protein 17, Ccs: copper chaperone for Cu/Zn superoxide dismutase, Atox1: Antioxidant 1, MT: metallothionein, Atp7a: copper transporting alpha-polypeptide ATPase, Atp7b: copper transporting beta-polypeptide ATPase (Adapted from La Fontaine and Mercer, 2007; Lonnerdal, 2008).

When intracellular Cu levels elevate, Atp7a are sorted into vesicles that move toward the basolateral membrane to increase Cu efflux via exocytosis; and Atp7b sequesters Cu within vesicles and traffic to the apical membrane Cu excretion. In Cu-depleted cells, virtually all Atp7a is localized around the TGN with less than 3% found at the basolateral membrane; but, when exposed to high Cu concentrations, more Atp7a is observed in the cell periphery and with 8 to 10% at the basolateral membrane (Lonnerdal, 2008).

2.3.2 Copper transportation in blood

The majority of Cu excreted from the serosal intestinal cells is carried by various components of the blood to the liver (Hill and Spears, 2001). Albumin and macroglobulin/transcuprein are the two main carriers that immediately bind Cu ions upon entering portal blood (Linder et al., 1998; Liu et al., 2007). Albumin is the most abundant protein in plasma, it binds Cu ions with a high-affinity Cu binding site at the N-terminus and accounts for 10 to 15% of plasma Cu. Macroglobulin/transcuprein binds 2 Cu ions with higher affinity than albumin and accounts for 5 to 15% of plasma Cu (Linder, 2016). Copper bound to these carriers are cleared rapidly by the liver which is the major storage organ of Cu. Within the liver, Cu is distributed to various cell organelles, including mitochondria, microsomes, lysosomes, incorporated into a number of proteins, and the excess Cu will be excreted via bile in the feces (Cao, 1994).

Ceruloplasmin is one of the Cu-dependent proteins synthesized in the liver and acts as the main Cu binding glycoprotein in plasma, accounting for 40 to 70% of total plasma Cu (Linder, 2016). Ceruloplasmin is proposed to have functions in the oxidation of organic amines, Fe^{2+} oxidation and the regulation of cellular Fe levels, and radical scavenging, as

well as transporting Cu from the liver to all the other Cu-requiring tissues (Healy and Tipton, 2007).

2.3.3 Copper transport in placenta

Adequate Cu supplies are essential for normal fetal development (Gambling and McArdle, 2004). During pregnancy, many changes occur in Cu status of both mother and fetuses. Regarding humans, serum Cu levels during pregnancy increase steadily by about 1 fold from early to late gestation. Sow liver Cu contents increased by 2 fold from breeding to mid-gestation, and then decline after that. In contrast, Cu contents of the whole fetal body increased by about 12-fold from mid-gestation until farrowing in piglets (Figure 2.3). These results suggest that an enormous amount of Cu is transported from mother to fetuses during pregnancy. However, because of the high redox activity of Cu, Cu transfer between maternal and fetal circulations needs to be tightly regulated.

Placental syncytiotrophoblasts have a polarized epithelium with apical and basolateral membranes toward maternal and fetal circulation, respectively (La Fontaine and Mercer, 2007). It has been reported as the only cell in the placenta that expresses both ATP7A and ATP7B proteins (Hardman et al., 2004); therefore, placental syncytiotrophoblast is commonly used for human studies about Cu transfer between maternal and fetal circulation. however, it is still not clear how the two ATPases are linked to deliver Cu to the fetal circulation.

Figure 2.4 shows the proposed model of Cu homeostasis regulation in syncytiotrophoblast cells in the placenta during gestation. Uptake of Cu from maternal circulation is through Ctr1, which is the same high-affinity carrier as other polarized cells. Once transferred into syncytiotrophoblast cells, ATP7A and ATP7B drive Cu to different

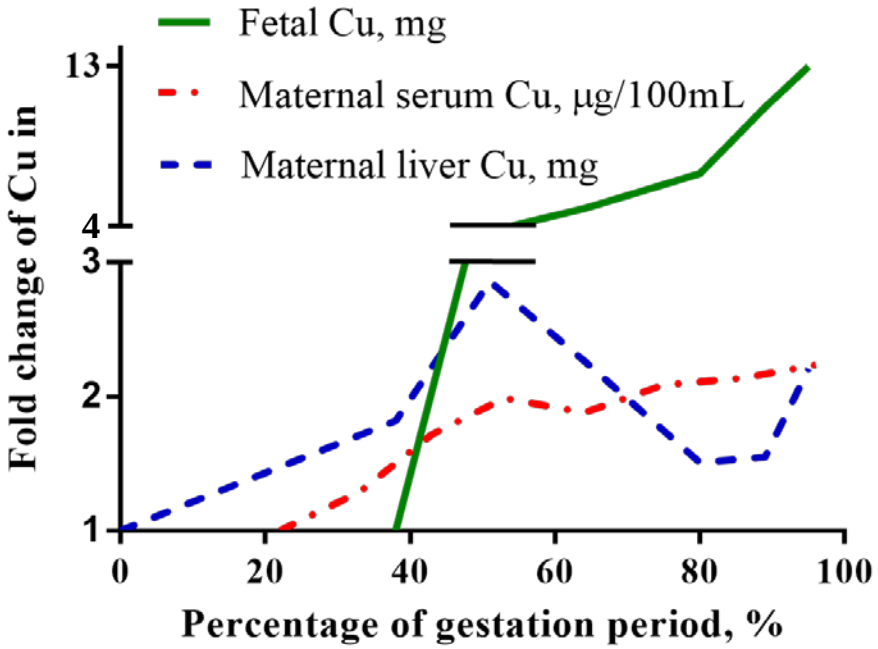

Figure 2.3. Fold change of copper concentrations or contents in serum and tissues. Solid green line: total fetal copper contents in piglets; dotted blue line: maternal liver copper contents in primiparous sow; dotted red line: maternal serum copper concentrations in human mother; percentage of gestation was calculated by (days of gestation on sampling day) ÷ (total days of gestation, 114 days for sow and 280 days for human) (adapted from Linder, 1991; Ma, 2011).

targets within the cell. Hardman et al. (2007) reported that both ATPases localized to the TGN but also differentially throughout the cytoplasm, with ATP7A located towards the basolateral surface for Cu delivery to fetal circulation, and ATP7B located towards the apical surface for excretion of excess Cu back to maternal circulation (Figure 2.4a). Because the Cu levels in maternal serum have been reported to be 5 times that in the umbilical cord, it was proposed that a role for ATP7B was to export Cu from the apical surface of the placenta as a protective mechanism to prevent excess Cu reaching the developing fetuses (Hardman et al., 2004). When insulin or estrogen levels increase, which

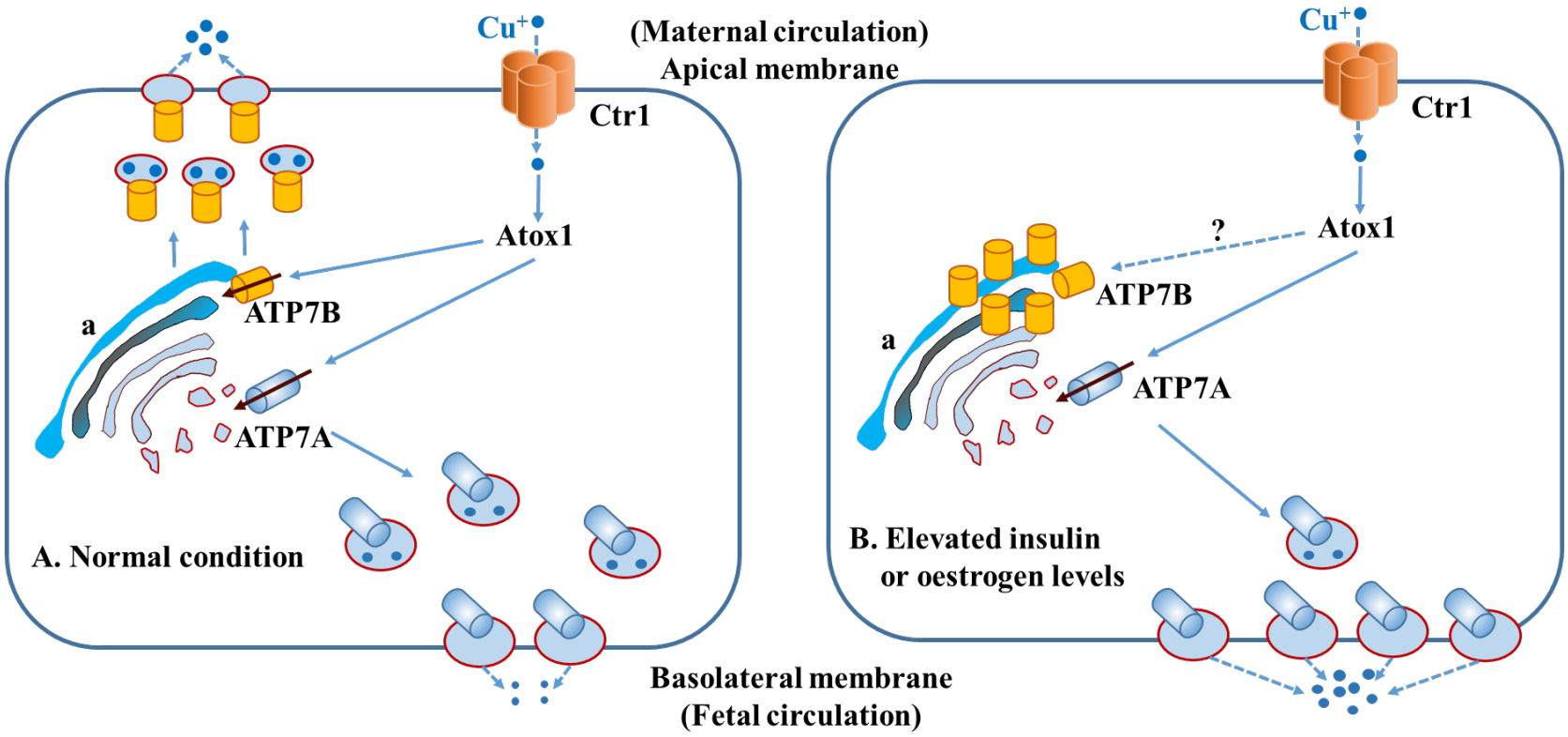

Figure 2.4. Proposed model of Cu homeostasis regulation in syncytiotrophoblast cells in placenta under (A) normal condition and (B) elevated insulin or estrogen levels. a: trans-golgi network, Ctr1: copper transporter 1, Atox1: Antioxidant 1, ATP7A: copper transporting alpha-polypeptide ATPase, ATP7B: copper transporting beta-polypeptide ATPase (Adapted from La Fontaine and Mercer, 2007; McArdle et al., 2008).

normally happened during the third trimester, substantial amounts of nutrients were deposited in fetuses (Figure 2.4b), resulting in a up-regulation of ATP7A and redistribution to the basolateral membrane to enhance the capacity of delivering Cu to fetal circulation. However, ATP7B is down-regulated in response to higher insulin and estrogen, and re-localizes to the TGN for adequate Cu supplies to the fetuses. Unfortunately, no result has been reported on how Cu regulation in syncytiotrophoblast cells responds to elevated Cu concentrations in maternal circulation as far as the author is aware of.

2.3.4 Copper transport in mammary gland

Copper delivery to milk is vital for neonatal development. Mice with a defective Atp7b gene due to a point mutation resulted in Cu accumulation in the mammary gland but deficiency in milk, thus yielded “toxic milk”. Rauch (1983) reported that toxic milk (*tx*) mice pups died in the second week after birth due to severe Cu deficiency. It has been demonstrated that Cu transportation in the mammary gland is regulated by prolactin through alternations in Ctr1 and Atp7a, which manipulate Cu uptake into the gland and secretion into milk (Kelleher and Lonnerdal, 2006; Lonnerdal, 2007).

The mechanisms governing the Cu delivery to milk are only now beginning to be revealed. According to the studies in humans and mice, ATP7A/Atp7a and ATP7B/Atp7b are critical proteins for intracellular Cu homeostasis regulation in the mammary gland (Ackland et al., 1999; Michalczyk et al., 2000). La Fontaine and Mercer (2007) proposed that ATP7A expression is up-regulated and relocalized from the TGN to the basolateral membrane to secrete Cu back to maternal circulation in the lactating mammary gland; whereas ATP7B expression is not altered, but the protein redistributes from the TGN toward the apical membrane where it may play a role in secreting Cu into milk. Llanos et

al. (2008) concluded that ATP7A might serve a protective role in mammary gland to export excess Cu back into the circulation rather than secrete it to milk.

2.3.5 Copper excretion and its regulation

The liver is the most important organ in maintaining whole-body Cu homeostasis because more than 95% of the Cu excretion is via bile and less than 5% via the urine under normal physiological conditions (Roberts and Sarkar, 2008). Hepatocytes are polarized cells with an apical (canalicular) domain, which is equipped with many different transport proteins responsible for the cellular elimination of Cu; as well as a basolateral (sinusoidal) domain with Cu import transporters (Wijmenga and Klomp, 2004). Figure 2.5 shows the network of proteins involved in Cu excretion and the proposed model of how Cu is trafficking within hepatocytes. Uptake of Cu from blood involves reductase at basolateral membrane because Cu associated with plasma carriers needs to be reduced from a divalent to monovalent form. After Cu ions are imported to the cytoplasm by Ctr1, they will be deployed to Cu chaperone proteins. Divalent cation transporter 1 was also found to be expressed in hepatocytes and may play a limited role in importing Cu from blood (Roberts and Sarkar, 2008). Similar to Cu trafficking in the enterocyte, several Cu chaperones, which include Atox1, Cox17, and Ccs, deliver Cu to different specific target molecules in the cytoplasm. Prohaska (2008) concluded that Atp7b was predominantly expressed in hepatocytes, but that Atp7a was not expressed in adult liver. Therefore, Atp7b is primarily involved in hepatic copper homeostasis under various physiological conditions. When intracellular Cu concentrations are low or normal, Atp7b, which resides at the TGN, receives Cu from Atox1 and incorporates it into holoceruloplasmin and eventually exports it into the blood. When intracellular Cu concentrations are elevated, Atp7b is rapidly

translocated from the TGN to a vesicular compartment diffusely localized within the cell and expedites biliary excretion of Cu (Hung et al., 1997; Wijmenga and Klomp, 2004; Roberts and Sarkar, 2008). In weanling pigs, hepatic mRNA expression of *Atp7b* has been reported to have significant elevation ($P < 0.05$) when 225 mg/kg of Cu as either TBCC or $CuSO_4$ were supplemented to the diets, and the increased expression might be associated with enhanced Cu excretion (Huang et al., 2015)

The process of secreting Cu from hepatic cells to bile canaliculus may also involve a protein named Cu metabolism gene MURR1-containing domain 1 (Commd1) (Roberts and Sarkar, 2008). The functions of Commd1 are proposed to regulate the protein stability of

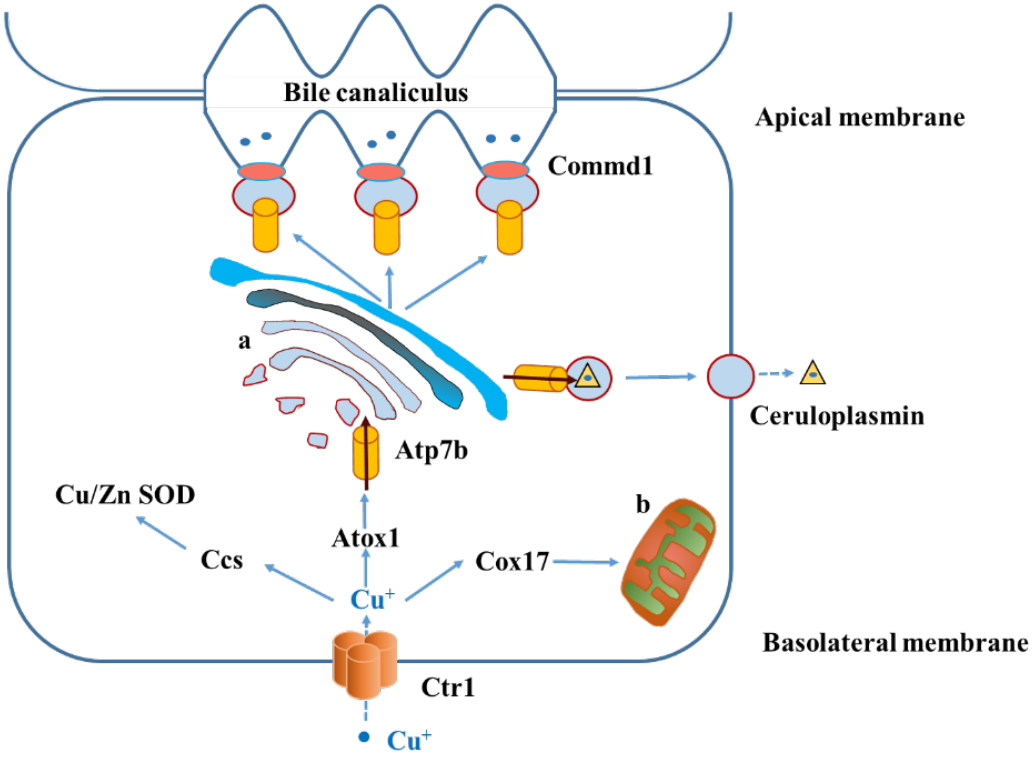

Figure 2.5. Proposed model of copper trafficking in the hepatocyte. a: trans-golgi network, b: mitochondria, Ctr1: copper transporter 1, Cox17: cytochrome c oxidase assembly protein 17, Ccs: copper chaperone for Cu/Zn superoxide dismutase, Atox1: Antioxidant 1, MT: metallothionein, Atp7b: copper transporting beta-polypeptide ATPase (Adapted from Roberts and Sarkar, 2008; Wijmenga and Klomp, 2004).

Atp7b, and it has been demonstrated to be critical at the final step to excrete Cu into the bile canaliculus (Fedoseienko et al., 2014). Copper excreted into bile is in the form of a complex that is not available for reabsorption (Tao and Gitlin, 2003).

2.3.6 Biomarkers for evaluating copper status of animals

Serum or plasma concentrations of Cu and Cp are the most often used indicator of evaluating Cu status for pigs because they are tightly controlled and only decreased in moderate or severe Cu deficiency (Milne, 1998). Ceruloplasmin is an acute-phase response protein that increases during inflammatory infections (Hill and Spears, 2001), which is a common situation in intensive pig production. Therefore, Cp may not be an ideal marker for Cu excess in pigs. Furthermore, Cp binds between 6 and 8 Cu ions per molecule and accounts for 95% of Cu in plasma (Harris and Gitlin, 1996; Bielli and Calabrese, 2002). Thus, the limitation of Cp may apply to serum or plasma Cu, because serum Cu is a reflection of Cp (Danzeisen et al., 2007).

Liver Cu content is widely used as the criterion to determine the relative bioavailability of Cu in different forms for various animal species (Ward et al., 1996; Miles and Henry, 2000; Guo et al., 2001). It has been thought as a reliable indicator of Cu status, but collecting liver tissue requires either specialized techniques of biopsy or euthanization, which may be impractical for on-farm pig production environments.

Hair was thought, at one time, to be a potential biopsy material for trace mineral status evaluation. Because sampling hair is relatively safe and easy, and the trace minerals in hair are more concentrated and stable than in other tissues or body fluid (Hopps, 1977). Kellaway et al. (1978) reported that progressive changes in liver Cu levels were closely paralleled with changes in hair Cu levels in steers, and the relationship between Cu levels

in liver and hair was shown to be asymptotic. The hair Cu concentrations of neonatal pigs were found to be much higher than that of sows, which might be due to the high concentration of Cu stored in the liver of fetal pigs; consequently, neonatal hair Cu contents may not be necessarily representative of the overall Cu status (Friendship et al., 1985).

The measurement of the activity of several cuproenzymes was used to evaluate copper status in both animals and humans. Erythrocyte Cu/Zn SOD was reported to have a decreased activity when experimental subjects were Cu deficient (Uauy et al., 1997). However, similar to Cp, SOD is not responsive to Cu excess. Moreover, the fact that SOD is an acute-phase protein may be confounded in the relationship between SOD activity and Cu status of animals (Danzeisen et al., 2007).

In recent years, some intracellular Cu chaperone proteins have been suggested as potential biomarkers for Cu status because of their sensitivity and specificity to both Cu deficiency and overload (Harvey and McArdle, 2008). The most promising candidate for accurate and sensitive detection is CCS, which delivers intracellular Cu to the metal-binding site of Cu/Zn SOD (Danzeisen et al., 2007). It has been reported in rats that erythrocyte CCS protein expression increased during Cu deficiency and declined with Cu overload when compared with rats with normal Cu status (Bertinato et al., 2003; Bertinato et al., 2010).

2.4 Pharmacological levels of copper and swine nutrition

NRC (2012) recommends 3 to 6 mg/kg of dietary Cu for growing pigs and 10 to 20 mg/kg for reproducing females. In contrast, it is a common practice to supplement much higher levels of Cu in the nursery and growing phases in modern pig production. Barber et al. (1957) firstly reported that pharmacological concentrations of Cu (125 to 250 mg/kg)

from $CuSO_4$ exerted growth-enhancing effects on finishing pigs. Moreover, in the next decades, a number of studies demonstrated that dietary supplementation of 125 and 250 mg/kg of Cu from $CuSO_4$ effectively promoted growth performance of pigs. Unfortunately, the mode(s) of action of pharmacological levels of Cu is still elusive. In recent years, concerns about using pharmacological levels of Cu in swine diets has become more ubiquitous. Since dietary Cu is poorly absorbed by pigs, consequently a large amount of Cu has been excreted to the environment and may cause some pollution issues. Therefore, more effects have been put on finding a more efficient and safer source of Cu, as well as to reveal the underlying mechanism(s) of pharmacological levels of Cu.

2.4.1 Copper digestibility of swine diets

Digestibility of dietary Cu is rarely evaluated in pigs. Table 2.5 summarizes the results of Cu digestibility for pigs and sows from peer-reviewed journals since 1994. Regarding growing pigs, when dietary Cu level was lower than 70 mg/kg, 14 out of 15 observations showed that the apparent total tract digestibility (ATTD) of Cu was between 20 to 60%. However, the studies with Cu levels greater than 70 mg/kg reported the ATTD of Cu to be less than 15%. This is in agreement with McDowell (2003b), who stated that Cu digestibility was likely to be greater in Cu-deficient than Cu-sufficient diets. In addition, the Cu digestibility of gestating sows that received 2 mg/kg of Cu was at least 50% higher than that of sows that received 12 mg/kg of Cu during early, middle, and late gestation (Cao and Chavez, 1995b).

Regarding the studies that reported lower Cu digestibility (lower than 15%) in growing pigs (Apgar and Kornegay, 1996b; Deng et al., 2010), dietary Fe, Mn, and Zn levels were

Table 2.5. Effects of copper sources and levels on apparent total tract digestibility (ATTD) of dietary copper in pigs and sows[1, 2]

Body weight, kg[3]	Copper source	Dietary trace mineral levels, mg/kg				ATTD of Cu, %	Cited references
		Copper[4]	Iron	Manganese	Zinc		
Growing pigs							
40.2	Copper sulfate	4	103	13	20	31.8	Liu et al., 2014
18.5	Copper sulfate	8	98	16	29	35.4	Veum et al., 2009
19.9	Copper sulfate	10	118	16	49	28.0	Veum et al., 2009
20.8	Copper sulfate	12	138	16	69	23.8	Veum et al., 2009
20.6	Copper sulfate	14	158	16	89	22.0	Veum et al., 2009
21.2	Copper sulfate	16	178	16	109	19.8	Veum et al., 2009
44.9	Copper sulfate	20	178	50	172	23.7	Korniewicz et al., 2007a
44.9	Copper -Yeast	20	178	50	172	28.1	Korniewicz et al., 2007a
46.9	Copper sulfate	20	178	50	172	21.9	Korniewicz et al., 2007b
11.7	Copper-Proteinate	20	100	50	40	21.2	Deng et al., 2010
30.6	Copper-Proteinate	24	100	46	137	58.8	Lebel et al., 2014
30.8	Copper sulfate	25	100	46	144	43.8	Lebel et al., 2014
81.4	Copper sulfate	36	350	120	300	11.9	Apgar and Kornegay, 1996
40.2	Copper sulfate	63	201	32	57	37.1	Liu et al., 2014
40.2	Copper-$(HMTBa)_2$	64	207	33	60	50.5	Liu et al., 2014
11.7	Copper sulfate	180	250	50	180	12.1	Deng et al., 2010
83.7	Copper-Lysine	245	350	120	300	8.8	Apgar and Kornegay, 1996
81.4	Copper sulfate	250	350	120	300	12.0	Apgar and Kornegay, 1996

Continued

Table 2.5 continued

Gestating sows							
d 30 to 35	-	2	452	42	71	12.4	Cao and Chavez, 1995
d 30 to 35	Copper carbonate	12	460	42	70	2.5	Cao and Chavez, 1995
d 60 to 65	-	2	452	42	71	17.5	Cao and Chavez, 1995
d 60 to 65	Copper carbonate	12	460	42	70	9.1	Cao and Chavez, 1995
d 90 to 95	-	2	452	42	71	31.1	Cao and Chavez, 1995
d 90 to 95	Copper carbonate	12	460	42	70	20.9	Cao and Chavez, 1995

[1]All the studies summarized in this table used corn-SBM diets except for Cao and Chavez (1995b) who used semi-purified diets, Korniewicz et al. (2007a) and Korniewicz et al. (2007b) who used wheat-based diets, and Veum et al. (2009) who used barley-SBM diets.

[2]All the studies summarized in this table used the direct method (total collection of feces) to determine apparent total tract digestibility of dietary copper, except for Veum et al. (2009) who used Chromium(III) oxide as an indigestible indicator.

[3]Body weight refers to the weight of pigs at the beginning of the collection.

[4]Copper level comprises the copper from both feed ingredients and extra supplementation.

much higher than the other studies in the table. Elevated dietary levels of Fe, Mn, and Zn have been demonstrated to have adverse impacts on Cu bioavailability for animals. Hill et al. (1983a; 1983b) reported that gilts that fed 5,000 mg/kg of Zn for over a year resulted in declined liver Cu concentrations and increased kidney Cu concentrations, and their subsequent offsprings developed signs of Cu deficiency. Adeola et al. (1995) also demonstrated that 100 mg/kg of dietary Zn significantly depressed Cu absorption when dietary Cu level was 14 mg/kg for growing pigs. Iron is thought to be an antagonist in Cu absorption and utilization. Hedges and Kornegay (1973) reported that dietary Fe at 312 mg/kg dramatically decreased Cu contents in liver and kidney when 257 mg/kg of Cu was present in diets compared to diets containing 101 mg/kg Fe. Some recent studies in cattle indicated that high dietary levels of Mn might depress duodenal expression of Dmt1, which imports Fe and Cu from the intestinal lumen to enterocytes, and subsequently affect Cu absorption and Cu status of cattle (Hansen et al., 2009; Hansen et al., 2010).

Regarding the growing pig studies only, there are 5 observation in this table derived from organic sources of Cu that resulted in an average ATTD of 33.5%, which suggested an improvement of 34% when compared to the studies with inorganic sources of Cu (average ATTD of 24.9%). Many studies have demonstrated that adding organic trace minerals in growing-finishing diets could enhance absorption and decrease fecal excretion of trace minerals, as well as improve their deposition in various tissues (Creech et al., 2004; Balfagón-Romeo, 2006; Huang et al., 2010a; Liu et al., 2014). Furthermore, dietary supplementation of organic trace minerals for reproducing sows has been reported to enhance reproductive performance and trace mineral concentrations in milk (Mahan and Peters, 2004; Peters and Mahan, 2008; Peters et al., 2010). Richards et al. (2010) suggested

that the antagonisms that occurs among different minerals and with phytic acid might be reasons for the low bioavailability of inorganic trace minerals; whereas organic trace minerals may minimize such antagonisms, and thus result in an improved bioavailability (Peters and Mahan, 2008).

2.4.2 Sources of copper in swine diets

The growth promoting effects of pharmacological levels of Cu from $CuSO_4$ have been extensively evaluated during the past decades. Cromwell (2001) summarized that nursery and growing pigs that received high Cu diets (200 to 250 mg/kg) with $CuSO_4$ had an average improvement of 11.9 and 6.9% in ADG, as well as 4.5 and 3.6% improvement in Feed/gain ratio, when compared to pigs that were fed diets containing 6 to 12 mg/kg of Cu. Therefore, this section will only discuss the other sources of Cu that are also widely used in swine diets.

2.4.2.1 Tribasic copper chloride

A total of 4 inorganic sources of Cu are listed in NRC (2012), which are $CuSO_4$, TBCC, copper oxide (CuO), and copper carbonate ($CuCO_3$). Copper oxide has been reported to be largely biologically unavailable for animals in different species (Cromwell et al., 1989; Baker et al., 1991; Kegley and Spears, 1994). Miles and Henry (2000) concluded that the bioavailability of $CuCO_3$ was intermediate between CuO and $CuSO_4$ for pigs. In contrast, many studies have demonstrated that TBCC was at least equally effective to promote growth performance and enhance tissue deposition for pigs when compared with $CuSO_4$ (Cromwell et al., 1998; Shelton et al., 2011; Fry et al., 2012; Coble et al., 2015).

Tribasic copper chloride is a more concentrated form of Cu than $CuSO_4$ (58 vs. 25% of Cu). It occurs in 3 forms, which are α- and β-crystals, as well as the amorphous form. An

in vitro study showed that multi-step incubation in the environment stimulating pH values of stomach, small intestine, and large intestine, respectively, resulted in similar solubility ($P > 0.05$) of Cu from $CuSO_4$ ($CuSO_4 \cdot H_2O$ and $CuSO_4 \cdot 5H_2O$) and from TBCC (α-form, β-form, and a α- and β- mixed form) (Park and Kim, 2016).

Shelton et al. (2011) reported that adding 150 mg/kg of TBCC in nursery diets increased overall ADG and ADFI ($P < 0.01$) when compared to diets without supplemental Cu. Cromwell et al. (1998) conducted 3 experiments involving a total of 915 nursery pigs and demonstrated that pharmacological levels (100 to 200 mg/kg) of Cu as TBCC is as effective as $CuSO_4$ in improving growth in weanling pigs. Moreover, some recent studies showed that weanling pigs fed diets supplemented 225 mg/kg of Cu from either TBCC or $CuSO_4$ for 12 or 33 days yielded similar growth performance (Fry et al., 2012; Huang et al., 2015). Carpenter et al. (2016b) reported no difference in overall growth performance between nursery pigs that were fed with similar levels (75 to 225 mg/kg) of Cu from either TBCC or 2-hydroxy-4-methylthio butanoic acid chelated Cu ($Cu(HMTBA)_2$).

It has been reported that 150 mg/kg of supplemental Cu from TBCC increased ADG of pigs from 30 to 80 kg, but did not affect growth performance from 80 to 130 kg as compared to pigs fed diets without Cu supplementation; moreover, the elevated dietary Cu level did not alter carcass characteristics (Coble et al., 2014; Coble et al., 2015). Coble et al. (2013a) demonstrated that finishing pigs showed a preference for the TBCC diet over the $CuSO_4$ diet (150 mg/kg) in a 15-day feed preference study ($P = 0.01$); unfortunately, results from growth trials showed that ADFI of pigs fed with TBCC diets did not differ from those fed with $CuSO_4$ diets in nursery and growing-finishing phases (Cromwell et al., 1998; Coble et al., 2013b).

Ionic Cu is thought of as a prooxidant in swine diets and also in the body, and oxidative damage has been linked to chronic Cu overload or exposure to excess Cu (Gaetke and Chow, 2003; Huang et al., 2015). It has been reported that the duodenal malondialdehyde (MDA) levels significantly increased ($P < 0.05$) in nursery pigs that were fed diets with 225 mg/kg of added Cu from TBCC or $CuSO_4$ compared to a control group (6.7 or 14.3 mg/kg of Cu in diet); however, TBCC group pigs had numerically lower MDA levels than that in the $CuSO_4$ group pigs (Fry et al., 2012; Huang et al., 2015).

2.4.2.2 Copper-amino acid chelate/complex

Since pigs have a low absorption rate of inorganic Cu, elevated dietary Cu from inorganic sources inevitably results in greater excretion of Cu into the environment and consequently may lead to environmental pollution issues (Roof and Mahan, 1982). Copper-amino acid (Cu-AA) chelate/complex is thought to be a more bioavailable source of Cu, because it is proposed to be absorbed in the animal through amino acid uptake mechanisms rather than ion uptake mechanisms in the intestine (Ashmead, 1993), and thus has a reduced incidence of antagonism with other dietary constituents in the gastrointestinal tract (Wang et al., 2007; Zhao et al., 2010).

Copper-lysine complex has been demonstrated to have 124% relative bioavailability to $CuSO_4$ in chicks using liver Cu concentration as an index (Guo et al., 2001). However, the relative bioavailability of Cu-lysine complex in swine has not been reported. Coffey et al. (1994b) conducted 8 experiments involving 1,301 nursery pigs to evaluate the efficacy of a Cu-lysine complex compared to $CuSO_4$, the results showed that pigs that received the Cu-lysine complex had numerically greater percentage improvements in ADG and ADFI with increasing Cu levels (0, 100, and 200 mg/kg of supplemental Cu) over $CuSO_4$ group

pigs; however, when dietary Cu concentration was 200 mg/kg, pigs that were fed $CuSO_4$ had significantly higher liver Cu concentrations ($P < 0.025$) than the Cu-lysine complex group pigs. In contrast, Apgar et al. (1995) reported that growth performance of weanling pigs was not affected by the sources of dietary Cu (Cu-lysine complex and $CuSO_4$), but that the liver Cu concentration was significantly higher for the Cu-lysine complex group over the $CuSO_4$ group when the dietary Cu level was 200 mg/kg. Apgar and Kornegay (1996a) also reported that finishing pigs fed diets containing 200 mg/kg of Cu from Cu-lysine complex and $CuSO_4$ absorbed and retained similar amounts of Cu in a balance study.

Copper methionine hydroxy analogue chelate [$Cu(HMTBa)_2$] is another Cu-AA chelate that is available in swine diets. Zhao et al. (2014) reported that pharmacological levels of Cu (150 or 170 mg/kg) from $Cu(HMTBa)_2$ significantly increased ADG and liver Cu concentration of nursery pigs compared to the $CuSO_4$ group that contained the same level of Cu. Jang et al. (2017a) also reported a greater Cu deposition in the liver for nursery pigs fed a diet containing 150 mg/kg of Cu from $Cu(HMTBa)_2$ than those fed a diet with 150 mg/kg of Cu as $CuSO_4$. A recent meta-analysis by Ma et al. (2015) from 6 nursery trials conducted under commercial conditions showed that the linear slope for increasing Cu supplementation on G: F was 2.1 fold higher for $Cu(HMTBa)_2$ than that of $CuSO_4$ in 5 to 25 kg pigs. However, 134 mg/kg of supplemental Cu from $Cu(HMTBa)_2$ or $CuSO_4$ have been reported to yield similar growth performance ($P > 0.05$) for growing pigs (Huang et al., 2010a; Huang et al., 2010b).

2.4.2.3 Copper proteinate

Copper proteinate was thought to be more bioavailable than inorganic Cu sources because they were assumed to be uptaken by peptides transporters in the intestine

(Ashmead, 1993). Aldridge (2008) demonstrated that Cu absorption from Cu proteinate was decreased when peptide transporter 1 (PepT1) was blocked, while PepT1 inhibitors did not affect Cu absorption from $CuSO_4$ in jejunal tissues harvested form postweaning pigs. Veum et al. (2004) reported that weanling pigs fed 100 mg/kg Cu as Cu proteinate absorbed and retained more Cu and excreted less Cu ($P \leq 0.003$) than pigs fed 250 mg/kg Cu as $CuSO_4$, although no difference was observed in growth performance. When Cu was supplemented at a low level (10 mg/kg), growing barrows fed the Cu proteinate diet had greater ATTD and retention of Cu ($P < 0.05$) than pigs fed the $CuSO_4$ diet (Lebel et al., 2014). Moreover, Deng et al. (2010) reported that nursery pigs fed 20 mg/kg Cu as Cu proteinate maintained the same growth rate as pigs fed 180 mg/kg Cu as $CuSO_4$, but Cu proteinate group absorbed a greater amount of Cu and had significantly higher retention rate than the $CuSO_4$ group ($P < 0.05$). Regarding sows, Yen et al. (2005) reported that top-dressing 14 mg of Cu as Cu proteinate per day on sow diet with 9 mg/kg Cu as $CuSO_4$ from day 108 of gestation until day 7 postweaning shortened weaning-to-estrus interval and improved the percentage of sows bred by day 7 postweaning ($P < 0.05$).

2.4.3 Proposed mechanisms of pharmacological levels of copper

Pharmacological levels (200 to 250 mg/kg) of Cu as $CuSO_4$ has been demonstrated to improve growth performance for nursery to finishing pigs, with the most pronounced effects during the nursery phase (Cromwell, 2001). A recent meta-analysis by Jongbloed et al. (2011) from 252 studies indicated that the supplementation of pharmacological levels of Cu were highly significant (linear and quadratic, $P < 0.01$) to improve growth rate and feed intake within the BW range between 5 to 25 kg; and the optimum Cu supplementation levels for growth rate and feed intake were 146 and 150 mg/kg, respectively. However, the

mechanisms for the growth-promoting effect of pharmacological levels of Cu in pigs are still elusive. It was hypothesized that the growth-stimulating action of pharmacological levels of Cu was attributed to its antimicrobial property (Fuller et al., 1960; Shurson et al., 1990a). In addition, it has also been proposed that pharmacological levels of Cu might exert systemic effects, which were independent of antimicrobial effects, and contributed to improved growth performance of pigs (Zhou et al., 1994a; Zhou et al., 1994b).

2.4.3.1 Antimicrobial effects

Copper had been used as an antimicrobial agent in human life for centuries until the advent of commercially available antibiotics in 1932 (Grass et al., 2011). In animal husbandry, high levels of Cu have been used in swine diets to enhance growth performance since the 1950s. Cromwell (2001) summarized 14 experiments and concluded that high dietary Cu (250 mg/kg) could improve growth performance of weanling pigs by a similar magnitude to those resulting from antibiotic feeding. Additionally, Shurson et al. (1990a) reported that a high Cu diet (283 mg/kg) reduced ADG and ADFI of germ-free pigs, but improved growth performance of conventionally reared pigs, when compared to the pigs fed the control diet (16 mg/kg).

High dietary Cu has been reported to influence intestinal microorganism populations that was measured via traditional viable count method, but the results were not consistent. Mei et al. (2010) indicated that higher dietary Cu levels (100, 175, and 250 mg/kg, as $CuSO_4$) tended to decrease the population of *Lactobacilli* and E*nterobacteriaceae* in cecal contents of nursery pigs. Wang et al. (2012) also reported that nursery pigs fed diets containing 100 mg/kg Cu as copper-loaded chitosan nanoparticles (CNP-Cu) significantly decreased the population of *Escherichia coli* in digesta samples obtained from duodenum, jejunum, ileum,

and caecum; and increased populations of *Lactobacillus* (jejunum and caecum) and *Bifidobacterium* (duodenum and caecum). However, low Cu supplementation (36 mg/kg) was reported to not alter microflora populations in the small intestine and proximal colon (Xia et al., 2005).

Because only a small percentage of microorganism species can be cultured, the viable count method inevitably underestimates microbial diversity in a community (Cangelosi and Meschke, 2014). In contrast, nucleic acid-based methods that emerged in recent years have greatly advanced the ability to detect diverse microorganisms independently of microbiological culture (Harwood and Buckley, 2007; Medini et al., 2008). Namkung et al. (2006) collected ileal and colonic digesta samples from nursery pigs fed diets with 15 or 250 mg/kg added Cu as $CuSO_4$ for 14 days, and then evaluated microbiota via polymerase chain reaction and denaturing gradient gel electrophoresis (PCR-DGGE) analysis of the 16S rRNA genes. Pigs fed high Cu diets had reduced number of bands in the DGGE gel compared to low Cu pigs in ileal and colonic digesta. Moreover, the similarity analysis showed a low degree of microbiota similarity (less than 40%) between high and low Cu fed pigs in ileal and colonic digesta. These results might suggest that pharmacological levels of Cu reduce the diversity of ileal and colonic microbiota. However, Perez et al. (2011) reported that the fecal samples of nursery pigs fed diets supplemented with 100 mg/kg Cu as Cu-AA or 250 mg/kg Cu as $CuSO_4$ generated a similar number of DGGE gel bands ($P = 0.87$) with those from basal diet group (without Cu supplementation); moreover, Sorenson's similarity percent showed no significant difference across treatments either. The discrepancy between the mentioned studies might be due to different types of

samples that were used for microbiota analysis (ileal and colonic digesta sample vs. fecal sample), or different nutrient levels (higher CP and SID lysine levels in the former study).

2.4.3.2 Systemic effects

Zhou et al. (1994a) conducted 2 experiments to examine the growth-promoting effects of parenterally administrated Cu on weanling pigs. After injecting pigs with increasing dosages of Cu histindinate every 2 days for 18 days (injection dosage was estimated by multiplying assumed daily Cu intake with 0, 5, 10, and 15% or 0, 2, 4, 6, and 8% apparent absorption coefficients of Cu), results showed that increasing Cu dosages increased ADG and serum mitogenic activity (quadratic, $P < 0.05$), elevated serum, liver, and brain Cu concentrations (linear, $P < 0.05$), and enhanced hepatic SOD activity (linear, $P < 0.05$). This clearly suggests a mode of action that does not involve antimicrobial activity exists. Furthermore, the same research group reported that pigs that had ad libitum access to diets containing 215 mg/kg Cu had significantly greater ADG and ADFI than ad libitum fed control pigs (8.2 mg/kg Cu), and pigs pair-fed the high Cu diets to the level of control pigs showed an intermediate ADG but the numerically greatest G:F; moreover, the Cu supplemented groups had increased serum mitogenic activity and pituitary growth hormone (GH) mRNA concentrations ($P < 0.05$) than the control group regardless of feeding method (Zhou et al., 1994b). These results indicate that the systemic effects of pharmacological levels of Cu are dependent on an increase in feed intake and growth regulatory system.

Feed intake stimulation

The hypothalamus is critical in food intake control because it is the place where various orexigenic and anorectic neuropeptides are synthesized and act on short- and long-term

dietary intake regulations. Furthermore, the hypothalamus plays a key role in sensing metabolic signals from peripheral organs and modulating feeding behaviors (Arora and Anubhuti, 2006; Yu and Kim, 2012). Neuropeptide Y (NPY) is a polypeptide that is primarily synthesized in the hypothalamus and plays a role in increasing food intake, decreasing thermogenesis, and regulating eating behavior (Kokot and Ficek, 1999). Li et al. (2008) reported that nursery pigs fed diets containing 125 and 250 mg/kg Cu as $CuSO_4$ resulted in improved ADG and ADFI ($P < 0.05$), as well as greater NPY concentrations and NPY mRNA expression ($P < 0.05$) in the hypothalamus compared to control pigs (10 mg/kg Cu). Zhu et al. (2011a) also reported that a dietary Cu ($CuSO_4$) level of 250 mg/kg increased NPY mRNA abundance ($P < 0.05$); and meanwhile reduced ($P < 0.05$) abundance of proopiomelanocortin and long-form leptin receptor, which were considered as anorexigenic proteins, in the hypothalamus of nursery pigs when compared to pigs that fed diets that contained 10 mg/kg of Cu as $CuSO_4$.

Ghrelin is secreted in the stomach and has been demonstrated to enhance appetite and increase food intake, promote GH release, and regulate energy homeostasis (Wren et al., 2001; Wu and Kral, 2004). Yang et al. (2012) reported that nursery pigs fed diets containing 125 mg/kg Cu as $CuSO_4$ or Cu methionine had improved ADG and ADFI, greater serum GH concentrations, and higher ghrelin mRNA expression in the fundic gland of the stomach ($P < 0.05$) compared to control pigs that received diets containing 5 mg/kg.

Growth regulation

Growth hormone is an anabolic hormone that induces positive nitrogen balance in intact animals and protein synthesis in muscle; and the effects of GH on nitrogen balance may be mediated by IGF-1 (Kostyo, 1968; Butler and Roith, 2001). It has been reported that serum

GH, IGF-1, and insulin levels of growing pigs increased when diets were supplemented with at least 100 mg/kg Cu as $CuSO_4$; and the increase in these hormones and proteins was in accordance with the increase of ADG (Wang et al., 2016). Yang et al. (2012) reported that nursery pigs fed diets containing 125 mg/kg Cu as $CuSO_4$ or Cu methionine showed an increased abundance of growth hormone-releasing hormone (GHRH) mRNA expression but a decreased abundance of somatostatin (SS) mRNA expression levels in the hypothalamus ($P < 0.05$) compared to the control group (5 mg/kg of dietary Cu).

When muscles become larger and stronger during growth, bones should adapt to increased loads imparted by muscles via adding mass, size, and strength (Cianferotti and Brandi, 2014). Copper is a cofactor for lysyl oxidase, which is involved in cross-linking collagen and plays a critical role in the functioning of connective tissue (Hill and Spears, 2001). Hill et al. (1983a) reported reduced lysyl oxidase activity in young Cu-deficient pigs. Moreover, GH has been demonstrated to stimulate longitudinal bone growth, and this effect was mediated via liver-derived IGF-1 (Butler and Roith, 2001). Therefore, the growth-promoting effects of pharmacological levels of Cu may partially be through enhancing bone development. Transforming growth factor-β originates from the local environment of bone tissues and is expressed in cartilage before calcification. It is thought to be a potent modulator of chondrocyte proliferation, differentiation, and extracellular matrix accumulation (Frenkel et al., 2000; McCormack et al., 2001). Wang et al. (2011a) and Zhu et al. (2011b) reported that greater Cu concentrations (15.6, 31.2, and 62.5 vs. 0 μmol/L) in chondrocyte cultures obtained from neonatal pigs resulted in increased DNA synthesis activity, greater IGF-1 and IGF-binding protein 3 contents, and enhanced transforming growth factor-β (TGF-β) gene expression.

2.4.4 Concerns of applying pharmacological levels of Cu in swine diets

The incorporation of 150 to 250 mg/kg Cu in swine diets largely exceeds the physiological requirements for pigs. Table 2.6 shows Cu requirements estimated by different organizations and the regulation of Cu usage in swine diets over the world. Currently, the maximum allowable contents of Cu in complete diets substantially exceed requirement estimates, even though the newly proposed maximum contents in the EU will dramatically reduce dietary Cu supplementation for young pigs from 170 to 25 mg/kg. Because of the poor digestibility of Cu that has been discussed before, a substantial amount of Cu in the diet is concentrated in the feces and eventually excreted to the environment, which includes spreading to arable lands as fertilizer (Grohskopf et al., 2016). Due to the high concentrations of Cu in the manure and low absorptive capacity of plants, long-term application of pig manure resulted in increase of Cu levels in the soil (Mattias et al., 2010). Furthermore, Anderson et al. (1991) reported that application of 1109 metric ton per hectare of Cu rich swine manure that contained an average of 1,316 mg/kg Cu over 11 years did not increase Cu concentrations in corn leaves and grains, or decrease corn yield.

Jensen et al. (2016) collected soil samples from 22 farms in Denmark, covering weaner and finisher pig production farms, as well as crop production only farms, for determination of Cu concentrations. The results showed that Cu concentration in the 0 to 25 cm and the 25 to 50 cm layers of soil increased from 7.9 and 6.1 mg/kg in 1986 to 11.7 and 9.4 mg/kg in 2014, respectively; which equals an annual increase by 1.7 and 1.6%. Meanwhile, Cu concentrations in Danish nature and forest soils are between 1.4 to 5.5 mg/kg. Furthermore, they also reported that Cu concentrations in the soil samples obtained from the 3 different types of farm were significantly different from each other ($P < 0.05$); with the greatest level

in the weaner farm (13.1 mg/kg), followed by the finisher farm (12.0 mg/kg), and lastly the crop farm (10.6 mg/kg). Copper exerts an innate antimicrobial property, and low concentrations of Cu (30 to 50 mg/kg) in soil have been reported to adversely affect soil microbial populations and activity, such as nitrogen fixation (McGrath et al., 1995). Additionally, elevated Cu and Zn concentrations in soil might also be phytotoxic for plants (Jondreville et al., 2003). Moreover, accumulated Cu in sediments from pig manure may leach out due to soil erosion, and consequently pose a risk to aquatic species (Jondreville et al., 2003; Jensen et al., 2016).

In addition to the issues of environmental pollution, high dietary Cu has been reported to induce specific Cu resistance in microorganisms as well. Amachawadi et al. (2011) reported that fecal *Enterococci* from piglets fed diets containing 125 mg/kg Cu exhibited greater overall prevalence of the *tcrB* gene, which is a conferred transferable Cu resistance gene, than those from low-Cu (16.5 mg/kg) fed piglets (21.1 vs. 2.8%); moreover, the minimum inhibitory concentration (MIC) for *tcrB*-positive was higher than that for *tcrB*-negative *Enterococci* (22.2 vs. 6.2 mM). Resistance to Cu in *Enterococci* is often associated with resistance to antimicrobial drugs like macrolides and glycopeptides, and such resistant bacteria may be transferred from the food-producing animals to humans via physical contacts or consumption (Yazdankhah et al., 2014).

Decreasing input of Cu into animal production is critical to minimize the output of Cu to the environment. Besides legislation, microbial phytase might be a promising way to improve Cu availability via freeing Cu from phytate; and organic sources of Cu, which are thought to be more bioavailable, could also be used in swine diets to decrease total dietary Cu levels.

Table 2.6. Recommendations and regulation of copper supply in swine diets[1]

Item	Recommendations of copper requirement, mg/kg					Regulation, mg/kg		
	NRC (2012)	PIC (2016)	MOA (2004)	INRA (1989)	ARC (1981)	European Union Current[2]	European Union New[3]	China[4]
Pigs								
3 to 30 kg	6.0 to 4.0	18.0 to 12.0	6.0 to 4.5	10.0	3.6	170	25	200
30 to 60 kg	4.0 to 3.5	12.0	4.5 to 4.0	10.0	3.6	170	25	150
60 to 90 kg	3.0	12.0 to 10.0	3.5	10.0	3.6	25	25	35
90 kg to market	3.0	10.0	-	10.0	3.6	25	25	35
Sows								
Gestation	10.0	15.0	5.0	-	-	-	25	35
Lactation	20.0	15.0	5.0	-	-	-	25	35

[1]The specific weight ranges listed within each nutrient requirement publications. The ranges listed for pigs encompass the low/high weights within all publications.
[2]Maximum contents of copper in swine complete feed that currently authorized by European Food Safety Authority.
[3]Maximum contents of copper in swine complete feed that newly proposed by European Food Safety Authority.
[4]Maximum contents of copper in complete swine feed according to Specification for Safe Use of Feed Additive that announced by Ministry of Agriculture of the People's Republic of China.

2.5 Conclusions

Longevity is critical to sow lifetime productivity, and thus important to achieve efficient and profitable pork production. With improving reproductive capacity due to continuous genetic selection, reproducing sows are correspondingly mobilizing more body reserve of nutrients, which includes the trace mineral Cu. Copper is an essential element for animals and plays various roles in metabolic processes. Pharmacological levels of Cu have been extensively studied in pigs and proved to enhance growth performance from weaning to growing phases effectively. The modes of action for pharmacological levels of Cu in pigs are hypothesized to be through antimicrobial properties in the intestinal lumen or systemic stimulation in brain or blood circulation.

The fact that sows are losing Cu stores in the body through repeated reproductive cycles, and the fetuses are depositing substantial amounts of Cu during gestation suggests that higher levels of Cu in breeding diets might be necessary to replenish the Cu reserves of sows and improve the Cu status of piglets (Mahan and Newton, 1995; Mahan et al., 2009; Ma, 2011). However, the number of studies evaluating pharmacological levels of Cu on reproductive performance of sows is limited. The influence of pharmacological levels of Cu in breeding diets on antioxidant status of sows and piglets has not been reported. Furthermore, effects of maternal dietary Cu levels on subsequent performance and health of progeny has not been reported either.

Therefore, the objective of the present research was to determine the effects of both dietary Cu sources and levels on performance and health of sows (Chapter 3), and the effects of dietary Cu levels on growth performance and response to immune challenge in nursery pigs derived from sows fed either high or low Cu (Chapter 4).

CHAPTER 3. Long-term Effects of Dietary Source and Level of Copper on Reproductive Performance, Antioxidant Status, Nutrient Digestibility, and Trace Mineral Deposition of Sows and Piglets

3.1 Abstract

The objective of the present experiment was to determine the long-term effects of dietary copper (Cu) source and level on performance and health of sows and their progeny. A total of 31 crossbred gilts (55 ± 2 d post-breeding; initial BW 189 ± 13 kg) were assigned to 1 of 6 dietary treatments in a 2 × 3 factorial arrangement with a completely randomized design. The first factor was 2 Cu sources [copper sulfate ($CuSO_4$) or tribasic copper chloride (TBCC)] while the second factor was 3 supplemental Cu levels (20, 120, or 220 mg/kg). All experimental diets were formulated to meet or exceed NRC (2012) nutrient requirement estimates for gestation and lactating sows. Sows continued on their respective dietary treatment throughout gestation and lactation until they were culled from the herd or until the weaning of the fourth litter. Fecal and blood samples were collected from sows at late gestation and lactation, and blood samples were collected from piglets at birth and weaning. In addition, colostrum and milk samples were collected during lactation. Upon completion of 3 or 4 parities, sows were slaughtered for tissue collection; and piglets were sacrificed at birth and weaning for organ harvest. Sows fed TBCC diets had significantly greater adjusted weaning weight for the litter and the individual piglet ($P < 0.10$), as well as adjusted lactation weight gain for the litter and the individual piglet ($P < 0.10$) when compared to sows that received $CuSO_4$ diets. Increasing dietary Cu level linearly increased ($P = 0.06$) live born piglet weight. Sows fed TBCC diets had lower apparent total tract digestibility (ATTD) of ether extract ($P = 0.01$) during late gestation, but greater ATTD of

dry matter, nitrogen, and phosphorous during lactation ($P < 0.05$). Increasing Cu levels linearly increased dry matter digestibility in lactating sows ($P = 0.02$). Milk from sows fed TBCC diets had a greater concentration of protein ($P = 0.02$) than that from sows fed $CuSO_4$ diets. Increasing Cu levels increased levels of milk fat and Cu (linear, $P < 0.05$); but linearly decreased lactose and Zn levels ($P < 0.05$). Lactating sows fed TBCC diets had a greater activity of Cu/Zn superoxide dismutase (SOD) and ceruloplasmin in serum than those fed $CuSO_4$ diets ($P < 0.05$). Increasing dietary Cu levels increased total and Cu/Zn SOD activity for lactating sows (linear, $P < 0.05$). Sows fed TBCC diets had lower concentrations of Cu ($P = 0.04$), but higher concentrations of iron and manganese ($P < 0.05$) in the liver, when compared to those fed $CuSO_4$ diets. In addition, liver Cu concentration significantly increased with increasing dietary Cu levels (linear and quadratic, $P < 0.05$). Increasing sow dietary Cu levels resulted in the elevation of concentrations and contents of Cu in the liver of weanling piglets (linear, $P < 0.0001$). Results of this experiment might suggest that TBCC is a superior Cu source compared to $CuSO_4$ regarding reproductive performance; high dietary Cu levels in sow diets did not have any apparent adverse effects on sows and progenies and resulted in greater birth weight of piglets.

Key Words: sows, tribasic copper chloride, copper sulfate, reproduction, antioxidant

3.2 Introduction

Sows are considered one of the most critical constituents of the swine industry because sow productivity determines the productive capacity of the swine herd; and genetic potential of the sows defines the maximum potential productivity of the entire system (Ball et al., 2008). Sow productivity of the major pork producing countries, which includes China, European Union, and the US, has increased by 11 to 28% from 2001 to 2013, regarding weaned or finished pigs produced per sow per year (Table 2.1). In addition, Koketsu et al. (2017) stated that the current target of the number of pigs weaned per sow per year is 30, and it is likely to increase to 40 in the future with the improvement of genetics and sow management. However, greater sow productivity inevitably leads to increased mobilization of minerals in body reserves, and reproductive capacity can be compromised if the mineral needs for reproductive demands exceed body stores and dietary intake (Mahan, 1990). It has been demonstrated that body mineral contents, including calcium, phosphorous, magnesium, potassium, sodium, aluminum, zinc, and Cu, decline in sows that complete three parities compared to those of similarly aged, nongravid gilts (Mahan and Newton, 1995).

Copper is required by pigs to serve many biological roles in the body, such as supporting iron metabolism, protecting tissues from oxidative damage, and maintaining immunity (Hill and Spears, 2001). The latest edition of the NRC estimates that the Cu requirement of growing pigs is 3 to 6 mg/kg and for gestating and lactating sows is 10 and 20 mg/kg, respectively (NRC, 2012). In addition, pharmacological concentrations of Cu (125 to 250 mg/kg) have been extensively studied and demonstrated to enhance growth performance of weanling and growing pigs (Cromwell, 2001). Furthermore, dietary supplementation of

pharmacological levels of Cu (250 mg/kg) in gestation and lactation diets from parity 1 to 6 has been demonstrated to increase Cu concentrations in sow liver and kidney, as well as improve piglet weight at birth and weaning (Cromwell et al., 1993). However, no study has been reported to assess the effects of high dietary Cu from different sources on reproducing sows.

Therefore, the objective of the present experiment was to determine the long-term effects of both dietary copper (Cu) source and level on performance and health of sows and their progenies.

3.3 Experimental procedures

This experiment was carried out in environmentally controlled rooms at the University of Kentucky Swine Research Center. The animal slaughter and sample collection were performed at the University of Kentucky Meats Science Laboratory. The experiment was conducted under protocols approved by the Institutional Animal Care and Use Committee of the University of Kentucky.

3.3.1 Animals, housing, management, and experimental design

A total of 31 eight-month-old gilts [Yorkshire; (Yorkshire × Landrace) × Duroc] were selected from a larger breeding group upon confirmation of their pregnancies through ultrasonic examination around 42 d post-breeding (SSD-500, Hitachi Aloka Medical, Ltd., Twinsburg, OH), and then allotted to the experiment at 55 ± 2 d post-breeding.

All gilts were provided with a common gestation diet supplementing 8 mg/kg Cu as $CuSO_4$ from breeding until allotted to a 2 × 3 factorial arrangement in a completely randomized design with initial BW and backfat depth balanced across treatments. The experimental factors included 2 Cu sources (TBCC and $CuSO_4$) and 3 supplemental Cu

levels (20, 120, and 220 mg/kg). All experimental animals received their respective treatment diet until they were removed from the experiment or until they weaned their fourth litter. The gilts had mean BW of 189 ± 13 kg and backfat depth of 22 ± 4 mm at the time of allotment.

After artificial insemination (2 services), gilts and sows were kept in individual gestation stalls (0.57 × 2.13 m^2) in an environmentally-controlled building with partially slatted concrete floors. On d 109 to 112 of gestation, they were moved to environmentally-controlled farrowing rooms and placed in farrowing crates (1.52 × 2.13 m^2) that were equipped with a plastic coated woven wire floor area, heating lamps and nipple waterer for piglets, as well as drinking nipple and feed trough for sows. Sows remained in the farrowing room until the end of the lactation period (20.4 ± 1.6 d). Once allotted to treatments, gilts and sows were floor-fed 1.9 kg/d of their gestation diet until farrowing; during the first 3 d after farrowing, they were provided with 3.2 kg/d of the lactation diets, and then increased gradually until daily feed intake reached at least 6.4 kg, thereafter sows were allowed to consume diets on an ad libitum basis during lactation. All the experimental animals had free access to water throughout the entire experiment.

Piglets were processed with teeth clipping, umbilical cord clipping and treating, tail docking, iron administration (1 mL of IRON-100 per piglet, Durvet, Inc., Blue Springs, MO), and ear notching within 24 h post-farrowing. No creep feed was offered to piglets during lactation, but access to sow's feed was not restricted. Each sow was intramuscularly injected with 1 mL of oxytocin (OXOJECT, Henry Schein Animal Health, Dublin, Ohio) and 4 mL of penicillin (PENJECT, Henry Schein Animal Health, Dublin, Ohio) in the

trapezius muscle on the farrowing day. Piglets and sows were, respectively, moved to gestation stalls and nursery rooms on weaning day.

Sows were exposed to boars starting on d 3 post-weaning until they were bred or had ample time to cycle. Sows were removed from experiment if they failed to rebreed or farrow, developed unsoundness, showed evidence of low milk production, became excessively thin or died. All sows that died were necropsied at the University of Kentucky Veterinary Diagnostic Laboratory (Lexington, KY).

3.3.2 Experimental diets

All experimental diets were formulated to meet or exceed NRC (2012) nutrient requirement estimates for gestation and lactating sows (Table 3.1 and 3.2). The gestation diets were formulated to contain 3,303 kcal/kg of ME, 15.42% of CP, and 0.80% of lysine; meanwhile, the lactation diets contained 3,298 kcal/kg of ME, 18.57% of CP, and 1.00% of lysine. No antibacterial agent was included in the gestation and lactation diets. Three trace mineral premixes containing different concentrations of Cu as TBCC were used for diet 1 to 3 to provide 20, 120, and 220 mg/kg of Cu, respectively; and 1 premix was used for diet 4 to 6 to provide 20 mg/kg of Cu as $CuSO_4$ in diets, and extra $CuSO_4$ was supplemented in diet 5 and 6 to reach dietary Cu levels of 120 and 220 mg/kg, respectively. Manganese and zinc provided by premix were in hydroxychloride form in diets 1 to 3, but in sulfate form in diets 4 to 6. The indigenous contributions of vitamins and trace minerals from the feed ingredients were disregarded. Titanium dioxide was used as an indigestible marker to determine ATTD of nutrients in gestation and lactation diets; it was added at a level of 0.30% by replacing an equal amount of corn. Table 3.3 demonstrates the average analyzed trace mineral concentrations in diets across different batches during the fecal

Table 3.1. Composition of gestation diets (as-fed basis)

Copper source:	Tribasic copper chloride			Copper sulfate		
Copper level, mg/kg:	20	120	220	20	120	220
Item Diet No.:	1	2	3	4	5	6
Ingredient, %						
Corn	76.47	76.47	76.47	76.47	76.43	76.39
Soybean meal, 48% CP	19.00	19.00	19.00	19.00	19.00	19.00
Grease, choice white	1.00	1.00	1.00	1.00	1.00	1.00
L-Lysine·HCl	0.06	0.06	0.06	0.06	0.06	0.06
Dicalcium phosphate	1.55	1.55	1.55	1.55	1.55	1.55
Limestone	1.00	1.00	1.00	1.00	1.00	1.00
Chromax[1]	0.05	0.05	0.05	0.05	0.05	0.05
Choline chloride[2]	0.10	0.10	0.10	0.10	0.10	0.10
Salt	0.50	0.50	0.50	0.50	0.50	0.50
Trace mineral premix[3]	0.15	0.15	0.15	0.15	0.15	0.15
Vitamin premix[4]	0.10	0.10	0.10	0.10	0.10	0.10
Santoquin[5]	0.02	0.02	0.02	0.02	0.02	0.02
Copper sulfate (pentahydrate)	0.00	0.00	0.00	0.00	0.04	0.08
Total	100.00	100.00	100.00	100.00	100.00	100.00
Calculated composition						
Metabolizable energy, kcal/kg	3,303	3,303	3,303	3,303	3,302	3,301
Crude protein, %	15.42	15.42	15.42	15.42	15.41	15.41
Lysine, %[6]	0.80	0.80	0.80	0.80	0.80	0.80

Continued

Table 3.1 continued

Methionine, %[6]	0.26	0.26	0.26	0.26	0.26	0.26
Methionine + Cysteine, %[6]	0.54	0.54	0.54	0.54	0.54	0.54
Threonine, %[6]	0.57	0.57	0.57	0.57	0.57	0.57
Tryptophan, %[6]	0.17	0.17	0.17	0.17	0.17	0.17
Calcium, %	0.84	0.84	0.84	0.84	0.84	0.84
Phosphorus, %[7]	0.37	0.37	0.37	0.37	0.37	0.37

[1]Chromax (Prince Agri-Products, Quincy, IL) provided 200 μg/kg of chromium to the final diet.

[2]Provided 600 mg/kg of choline chloride to the final diet.

[3]Supplied the following per kilogram of diets: 50 mg of Mn as manganese hydroxychloride for diet 1 to 3, as manganese sulfate monohydrate for diet 4 to 6; 100 mg of Fe as ferrous sulfate monohydrate; 125 mg of Zn as zinc hydroxychloride for diet 1 to 3, as zinc sulfate monohydrate for diet 4 to 6; 20, 120, and 220 mg of Cu as tribasic copper chloride for diet 1, 2, and 3; 20 mg of Cu as copper sulfate for diet 4, 5, and 6; 0.35 mg of I as calcium iodate; and 0.30 mg of Se as sodium selenite.

[4]Supplied the following per kilogram of diet: 11,000 IU of vitamin A; 1,100 IU of vitamin D3; 77 IU of vitamin E; 2.2 mg of vitamin K; 0.03 mg of vitamin B12; 8.25 mg of riboflavin; 27.50 mg of pantothenic acid; 30.25 mg of niacin; 4.95 mg of folic acid; 4.95 mg of vitamin B6; 1.65 mg of thiamin; and 0.36 mg of biotin.

[5]Santoquin (Monsanto, St. Louis, MO) supplied 130 mg/kg ethoxyquin to the final diet.

[6]Calculated composition of amino acids is presented on total basis.

[7]Calculated composition of phosphorus is presented on standardized total tract digestible basis.

Table 3.2. Composition of lactation diets (as-fed basis)

Copper source:	Tribasic copper chloride			Copper sulfate		
Copper level, mg/kg:	20	120	220	20	120	220
Item Diet No.:	1	2	3	4	5	6
Ingredient, %						
Corn	68.54	68.54	68.54	68.54	68.50	68.46
Soybean meal, 48% CP	27.00	27.00	27.00	27.00	27.00	27.00
Grease, choice white	1.00	1.00	1.00	1.00	1.00	1.00
L-Lysine·HCl	0.04	0.04	0.04	0.04	0.04	0.04
Dicalcium phosphate	1.60	1.60	1.60	1.60	1.60	1.60
Limestone	0.90	0.90	0.90	0.90	0.90	0.90
Chromax[1]	0.05	0.05	0.05	0.05	0.05	0.05
Choline chloride[2]	0.10	0.10	0.10	0.10	0.10	0.10
Salt	0.50	0.50	0.50	0.50	0.50	0.50
Trace mineral premix[3]	0.15	0.15	0.15	0.15	0.15	0.15
Vitamin premix[4]	0.10	0.10	0.10	0.10	0.10	0.10
Santoquin[5]	0.02	0.02	0.02	0.02	0.02	0.02
Copper sulfate (pentahydrate)	0.00	0.00	0.00	0.00	0.04	0.08
Total	100.00	100.00	100.00	100.00	100.00	100.00
Calculated composition						
Metabolizable energy, kcal/kg	3,298	3,298	3,298	3,298	3,296	3,295
Crude protein, %	18.57	18.57	18.57	18.57	18.56	18.56
Lysine, %[6]	1.00	1.00	1.00	1.00	1.00	1.00

Continued

Table 3.2 continued

Methionine, %[6]	0.30	0.30	0.30	0.30	0.30	0.30
Methionine + Cysteine, %[6]	0.62	0.62	0.62	0.62	0.62	0.62
Threonine, %[6]	0.69	0.69	0.69	0.69	0.69	0.69
Tryptophan, %[6]	0.22	0.22	0.22	0.22	0.22	0.22
Calcium, %	0.84	0.84	0.84	0.84	0.84	0.84
Phosphorus, %[7]	0.40	0.40	0.40	0.40	0.40	0.40

[1]Chromax (Prince Agri-Products, Quincy, IL) provede 200 μg/kg of chromium to the final diet.

[2]Provided 600 mg/kg of choline chloride to the final diet.

[3]Supplied the following per kilogram of diets: 50 mg of Mn as manganese hydroxychloride for diet 1 to 3, as manganese sulfate monohydrate for diet 4 to 6; 100 mg of Fe as ferrous sulfate monohydrate; 125 mg of Zn as zinc hydroxychloride for diet 1 to 3, as zinc sulfate monohydrate for diet 4 to 6; 20, 120, and 220 mg of Cu as tribasic copper chloride for diet 1, 2, and 3; 20 mg of Cu as copper sulfate for diet 4, 5, and 6; 0.35 mg of I as calcium iodate; and 0.30 mg of Se as sodium selenite.

[4]Supplied the following per kilogram of diet: 11,000 IU of vitamin A; 1,100 IU of vitamin D3; 77 IU of vitamin E; 2.2 mg of vitamin K; 0.03 mg of vitamin B12; 8.25 mg of riboflavin; 27.50 mg of pantothenic acid; 30.25 mg of niacin; 4.95 mg of folic acid; 4.95 mg of vitamin B6; 1.65 mg of thiamin; and 0.36 mg of biotin.

[5]Santoquin (Monsanto, St. Louis, MO) supplied 130 mg/kg ethoxyquin to the final diet.

[6]Calculated composition of amino acids is presented on total basis.

[7]Calculated composition of phosphorus is presented on standardized total tract digestible basis.

Table 3.3. The average analyzed trace mineral concentration (mg/kg; as-fed basis) in gestation and lactation diets across different batches during fecal collection period

Copper source:	Tribasic copper chloride			Copper sulfate		
Copper level, mg/kg:	20	120	220	20	120	220
Item Diet No.:	1	2	3	4	5	6
Gestation diets						
No. of observations	5	6	6	4	6	6
Copper	25	103	219	31	161	257
Iron	341	314	365	375	379	391
Manganese	100	76	76	86	88	86
Zinc	182	165	166	174	158	170
Lactation diets						
No. of observations	5	7	6	5	5	4
Copper	27	118	219	34	134	255
Iron	369	358	359	377	361	362
Manganese	107	83	77	90	92	89
Zinc	186	170	164	162	155	158

collection period (from August 2014 to December 2015). The analyzed trace mineral concentrations for each batch of diets are presented in Appendix 1 as Table A1.1.

3.3.3 Data and sample collection

3.3.3.1 Sow and litter performance

Gilts were weighed when allotted to dietary treatment. Both gilts and sows were weighed on the day they were moved from the gestation stalls to the farrowing crates for each lactation, within 24 h after farrowing, and on the weaning day. The amount of feed added and discarded were recorded every day throughout lactation.

The number of total piglets born, born alive, and stillborn were recorded within 12 h after farrowing; and the number of live piglets at weaning was also recorded. Individual piglet and litter weight were determined at birth and weaning. Because of different weaning

ages across litters, the weight of the piglet and litter at weaning were standardized to the end of 21-d lactation by the following equation modified from Jang et al. (2017b):

$$\text{Adjusted weight} = \text{Weight at birth} + \frac{(\text{Weight at weaning} - \text{Weight at birth})}{\text{Weaning age}} \times 21$$

3.3.3.2 Feed and fecal samples collection

This experiment started in March 2014 and ended in August 2016, so multiple batches of experimental diets were mixed. Representative samples of corn, SBM, and mixed feed were collected at the feed mill for every batch of experimental diets. Fecal samples from all sows were collected by grab sampling during their second and third parity; the daily collection periods were from d 98 to 102 of gestation and from d 15 to 17 of lactation. Feed and fecal samples were stored at -20°C until analyzed.

3.3.3.3 Blood sample collection

Blood samples from sows and piglets were collected by vena cava puncture with a syringe. Sow blood samples were collected at d 100 of gestation for the second and third parity, and at d 15 of lactation for the second to fourth parity. Regarding the third and fourth litters of each sow, one piglet with BW closest to litter average was selected for blood collection at birth. The sex of piglets selected for bleeding at birth was balanced within treatment by selecting piglets with opposite sex for the next litter of that treatment. In addition, one male and one female piglet with BW closest to litter average were selected for blood collection at weaning during the second to fourth litters of each sow. Blood samples at birth were collected within 12 h after farrowing; while weanling blood samples were collected on the weaning day. A total of 47 blood samples (24 males and 23 females) were collected from piglets at birth, and 107 blood samples (54 males and 53 females) were collected from piglets at weaning. After the blood collection, about 8 mL of whole blood

were transferred to a 16 × 100 mm vacutainer tube with polymer gel (Becton, Dickinson and Company, Franklin Lakes, NJ) for serum separation; and another 2 mL of whole blood was transferred to a 13 × 75 mm vacutainer tubes coated with K_2EDTA (Becton, Dickinson and Company, Franklin Lakes, NJ) for hematocrit (Htc) and hemoglobin (Hb) measurements. All blood samples were immediately placed on ice and then transported to the laboratory. Serum samples were obtained by centrifugation at 1700 × g for 20 minutes at 4°C; and then aliquoted into 1.5 mL Eppendorf Safe-Lock Tubes (Eppendorf North America, Hauppauge, NY), flash frozen in liquid nitrogen, and stored at −80°C until analyzed.

3.3.3.4 Colostrum and milk sample collection

During the third and fourth parity, colostrum and milk were respectively collected at 12 h post-farrowing and d 15 of lactation from each sow right after intramuscular (colostrum) or intravenous (milk) injection of 1 mL oxytocin (OXOJECT, Henry Schein Animal Health, Dublin, Ohio). Colostrum and milk samples were immediately placed on ice, aliquoted into 1.5 mL Eppendorf Safe-Lock Tubes (Eppendorf North America, Hauppauge, NY), and flash frozen in liquid nitrogen, and stored at −80 ° C until analyzed.

Milk yield of a 21-d lactation period was predicted by a Bayesian hierarchical model based on litter size and litter weight gain (Hansen et al., 2012). Since the predicted milk yield was in the gravimetric unit (kg), it has to be converted to the volumetric unit (L) by dividing milk density of each sample. Milk density was estimated by an equation that involved temperature (°C), milk fat content (%), milk protein content (%), and lactose and other solids (%) (Ueda, 1999).

3.3.3.5 Tissue sample collection

At the beginning of the experiment, 6 open gilts from the same breeding group with the experimental animals were slaughtered for tissue collection (liver, heart, right kidney, and both ovaries). After completing at least 3 parities, sows were weighed, stunned by electric shock, and killed by exsanguination. Then the abdominal cavity opened for collection of the tissues.

One piglet was sacrificed at birth, and another one was sacrificed at weaning for the third and fourth litter of each sow. On the farrowing day, one piglet with BW closest to litter average was euthanized by sodium pentobarbital (SOCUMB, Henry Schein Animal Health, Dublin, Ohio), liver, both kidneys, and heart were then collected. On the weaning day, another piglet with BW closest to the litter average and of the same sex with the one sacrificed at birth was euthanized for the same tissue collection. To obtain a balanced sex of sacrificed piglets within treatment, piglets sacrificed for the next litter of that treatment were of the opposite sex. A total of 46 piglets (23 males and 23 females) were sacrificed at birth, and 45 piglets (23 males and 22 females) were sacrificed at weaning. All the organs collected from sows and piglets were immediately weighed after being dissected from the body, placed on ice, and then transported to the laboratory and stored at −20°C until analyzed.

3.3.4 Sample processing and laboratory analysis

3.3.4.1 Apparent total tract digestibility

Fecal samples were thawed at room temperature overnight, pooled within sows and collection period, and then dried in a forced-air drying oven at 55°C for 1 wk. The dried fecal samples were air equilibrated, weighed, and ground through a 1 mm screen using a

Wiley Laboratory Mill (model 3; Arthur H. Thomas Co., Philadelphia, PA) for chemical analysis.

Feed and fecal samples were analyzed for dry matter (DM), gross energy (GE), ether extract (EE), nitrogen, Ca, P, titanium (Ti), Cu, Fe, Mn, and Zn. Dry matter was assessed according to the AOAC (1990) methods, involving overnight drying (105°C) of the samples in a convection oven (Precision Scientific Co., Chicago, IL). Gross energy contents were assessed by bomb calorimetry (model 1261 Isoperibol Bomb Calorimeter; Parr Instruments Co., Moline, IL). Nitrogen was measured using Dumas methodology in an automatic nitrogen analyzer (model FP-2000; LECO Corp., St. Joseph, MI). The ether extract was analyzed using Soxhlet extraction (method 920.39; AOAC, 1990).

About 4 g of feed samples and 1 g of fecal samples were placed in an ash furnace at 550°C for 3 h, and then dissolved in 40 mL of 1:3 hydrochloric acid/water on a hot plate that was preheated to 600°C. The solution was quantitatively transferred to 250 mL volumetric flask, brought to volume with deionized water and mixed thoroughly. Trace minerals (Cu, Fe, Mn, and Zn) and Ca were assessed by flame atomic absorption spectrophotometry (Thermoelemental, SOLAAR M5; Thermo Electron Corp., Verona, WI) according to a modification of an AOAC (1990) procedure (method 927.02 and 975.03B). Phosphorus was assessed by a gravimetric method (modification of method 968.08; AOAC, 1990). Titanium analysis was according to the method of Myers et al. (2004). The ATTD (%) of DM, GE, EE, N, Ca, P, Cu, Fe, Mn, and Zn in each diet were calculated according to the following equation modified from NRC (2012):

$$\text{ATTD (\%)} = [1 - \frac{\text{Nutrient}_{\text{feces}}}{\text{Nutrient}_{\text{feed}}} \times \frac{\text{Marker}_{\text{feed}}}{\text{Marker}_{\text{feces}}}] \times 100$$

The $Nutrient_{feed/feces}$ represents nutrient concentrations of feed and fecal samples, and the $Marker_{feed/feces}$ represents titanium dioxide concentrations of feed and fecal samples.

In addition, amounts of nutrients excreted in feces during late gestation and lactation were estimated with the following equation:

$$\text{Fecal excretion (g or mg/d)} = \frac{ADFI \times DM_{feed} \times (100 - ATTD_{DM}) \times Nutrient_{feces}}{100}$$

The ADFI during late gestation and lactation, DM of feed samples (DM_{feed}), the ATTD of DM ($ATTD_{DM}$), and nutrients concentrations in fecal samples ($Nutrient_{feces}$, DM basis) were used to estimate amounts of nutrients excreted through feces per day.

3.3.4.2 Blood, milk, and colostrum parameters

Malondialdehyde concentration, superoxide dismutase (SOD) activity, and Cp activity were analyzed in serum, colostrum, and milk samples. Colostrum and milk samples were centrifuged at 9,950 × g at 4°C for 10 minutes to separate fat from skim milk. After the fat layer was removed and discarded, the skimmed colostrum and milk samples were used for analysis. The analyzed results of colostrum and milk samples were converted to a whole milk basis by accounting for the analyzed fat content of each sample.

The content of the lipid peroxidation product MDA was determined using a commercial assay kit (Cayman, Ann Arbor, MI) according to the manufacturer's instruction. Total SOD activity was assessed by measuring the dismutation of superoxide radicals that were generated by xanthine oxidase and was determined by a commercial assay kit (Sigma, St Louis, MO) according to the manufacturer's instruction. Potassium cyanide (2 mM) was used to inhibit Cu/Zn SOD activity in the samples, and subsequently allowed for the measurement of Mn SOD activity with the same assay kit. The activity of Cu/Zn SOD was calculated as the difference between total and Mn SOD activity (Marklund, 1976). The

activity of Cp was determined according to the method of Schosinsky et al. (1974). Appropriate dilution factors were pre-determined for each assay, and all samples were tested in duplicate.

Colostrum and milk composition analysis were conducted using Foss Milkoscan™ FT 120 device (FOSS Electric, Eden Prairie, MN, USA) in the milk laboratory of the Division of Regulatory Services of the University of Kentucky. In addition, 2 mL of colostrum and milk samples were digested with nitric acid in a pressurized microwave digestion system (Mars 6, CEM Corporation, Matthews, NC) according to the manufacturer's program, and appropriately diluted. The digested solution was used to determine trace mineral concentrations by atomic absorption spectrophotometry (Thermoelemental, SOLAAR M5; Thermo Electron Corp., Verona, WI).

The whole blood samples collected in tubes containing anticoagulant as K_2EDTA were measured for Htc and Hb within 3 h after collection. Hematocrit was determined using standard hematocrit tubes and centrifugation, and Hb was measured using a commercial assay kit and Hb standard (Pointe Scientific, Inc., Canton, MI) according to the manufacturer's instruction.

3.3.4.3 Tissue trace mineral concentrations

Tissue samples from open gilts and sows were chopped into small pieces and then ground in a kitchen meat grinder (The butcher shop premium, KRUPS USA, Parsippany, NJ). Subsamples were weighed and put into a forced-air drying oven at 100°C until constant weight was reached, and then weighed again to determine DM. Tissue samples from piglets were chopped into small pieces and DM was determined using the same procedures as for

the sow tissue. After desiccation, a coffee grinder (Proctor Silex E160B, Hamilton Beach Brands, Inc., Washington, NC) was used to finely grind the samples.

About 0.5 g of finely ground tissue sample was digested with nitric acid in a pressurized microwave digestion system (Mars 6, CEM Corporation, Matthews, NC) according to the manufacturer's program, and appropriately diluted. The digested solution was used to determine trace mineral concentrations by atomic absorption spectrophotometry (Thermoelemental, SOLAAR M5; Thermo Electron Corp., Verona, WI). Total content of trace minerals for each collected organ were determined by multiplication of the wet-basis tissue trace mineral concentration and organ weight.

3.3.5 Statistical analysis

All data were subjected to ANOVA using the GLM procedure in SAS (Statistical Analysis System, Cary, NC, USA) for a completely randomized design. The individual sow and piglet served as the experimental unit and results are reported as least squares means.

The data of reproductive performance, ATTD of nutrients, as well as blood, colostrum, milk, and tissue parameters for sow and gilts were analyzed by a model that included 2 main effects as follows:

$$Y_{ijk} = \mu + S_i + L_j + parity_k + (S \times L)_{ij} + (S \times parity)_{ik} + (L \times parity)_{jk} + (S \times L \times parity)_{ijk} + e_{ijk}$$

Y = response variables (reproductive performance; ATTD of nutrients; antioxidant enzyme activity in serum, colostrum, and milk; hematocrit and hemoglobin; composition of colostrum and milk; organ weight; and tissue mineral concentrations)

μ = constant common to all observations

S_i = copper source

L_j = copper level

$parity_k$ = sow parity

$(S \times L)_{ij}$ = copper source × copper level

$(S \times parity)_{ik}$ = copper source × sow parity

$(L \times parity)_{jk}$ = copper level × sow parity

$(S \times L \times parity)_{ijk}$ = copper source × copper level× sow parity

e_{ijk} = error term of the model

The data of blood and tissue parameters for piglets at birth and weaning were analyzed by a model that included 2 main effects as follows:

$$Y_{hijk} = \mu + S_i + L_j + parity_k + sex_h + (S \times L)_{ij} + (S \times parity)_{ik} + (L \times parity)_{jk} + (S \times L \times parity)_{ijk} + e_{hijk}$$

Y = response variables (antioxidant enzyme activity in serum; hematocrit and hemoglobin; organ weight; and tissue mineral concentrations)

μ = constant common to all observations

S_i = copper source

L_j = copper level

$parity_k$ = sow parity

sex_h = piglet sex

$(S \times L)_{ij}$ = copper source × copper level

$(S \times parity)_{ik}$ = copper source × sow parity

$(L \times parity)_{jk}$ = copper level × sow parity

$(S \times L \times parity)_{ijk}$ = copper source × copper level× sow parity

e_{hijk} = error term of the model

A Chi-square test was used to analyze the distribution of the number of litters for gilts and sows. Orthogonal polynomial contrasts were performed to evaluate linear and quadratic effects of increasing dietary Cu levels. Statistical outliers were detected by Grubb's test outlier calculator (GraphPad Software, San Diego, CA, USA). The removed outliers are presented in Appendix 2. A post hoc statistical power analysis was conducted by G*Power (University of Düsseldorf, Dusseldorf, Germany) followed the instructions (Faul et al., 2007). The detailed statistical power analysis is presented in Appendix 3 as Table A.3.1. With the purpose of achieving appropriate statistical power and according to the result of the post hoc analysis, the α level used for determination of statistical significance was set at 0.10 for sow and litter performance data (α level at 0.15 for tendency), and set at 0.05 for all other data (α level at 0.10 for tendency).

3.4 Results

3.4.1 Sow and litter performance

A total of 106 litters were farrowed, with 52 litters from TBCC groups and 54 litters from $CuSO_4$ groups; 31, 38, and 37 litters were, respectively, from 20, 120, and 220 mg/kg Cu groups (Table 3.4). Chi-square analysis showed that there was no significant difference across dietary treatments. One sow from treatment 1 was culled because of reproductive failure (no litter farrowed), and another sow from treatment 3 was culled because of death from gastric torsion (after completion of 3 parities). The distribution of first- to fourth-parity litters was similar across treatments.

The effects of sow parity on sow and litter performance are presented in Appendix 4 as Table A.4.1. The first parity gilts had lighter BW at breeding, late gestation, farrowing, and weaning ($P < 0.01$), greater gestation BW gain and lactation BW loss ($P < 0.0001$), less lactation feed intake ($P < 0.01$), and smaller litter size and lighter litter weight at birth and weaning ($P < 0.05$), when compared to second- to fourth-parity sows. It should be noted that experimental diets were initiated around 55 d of gestation for gilts, whereas the multiparous sows had access to experimental diets throughout the entire reproductive cycle. Therefore, the reproductive performance data from gilts were excluded because of the potential qualitative differences between gilts and sows to affect the response. The results of reproductive performance for combined gilts and sows and gilts only are presented in Appendix 5 as Table A.5.1 and A.5.2.

Table 3.5 shows the effects of dietary Cu source and level on sow performance during the second- to the fourth-parity. Sow weights and weight changes were not affected by Cu source or Cu level ($P > 0.26$). However, sows fed TBCC diets tended to have increased lactation feed intake compared to sows fed $CuSO_4$ diets ($P = 0.13$). Furthermore, a Cu source × Cu level interaction was detected on lactation feed intake ($P = 0.02$), which exhibited a linear increase as increasing Cu levels within TBCC diets ($P = 0.03$) but a quadratic increase response within $CuSO_4$ diets ($P = 0.06$).

Sows fed the TBCC diets had more stillborn piglets ($P = 0.03$), greater adjusted weaning weight of piglet ($P = 0.07$), and adjusted litter ($P = 0.10$) and piglet ($P = 0.05$) weight gain than $CuSO_4$ fed sows. In addition, tendencies for greater litter weight of total born piglets ($P = 0.11$), piglet weight at weaning ($P = 0.13$), piglet weight gain during lactation ($P = 0.12$), and adjusted litter weight at weaning ($P = 0.14$) were observed for TBCC fed sows

compared to $CuSO_4$ fed sows. Litter performance (litter size, litter weight, and piglet weight) was not affected by Cu level (Table 3.6; $P > 0.17$); except for the weight of total piglets born ($P = 0.12$) or born alive ($P = 0.06$), which showed a linear increasing response to increasing dietary Cu levels. An interaction of Cu source and level was detected on adjusted piglet weight at weaning ($P = 0.06$), which showed a linear increase as increasing Cu levels within TBCC treatments ($P = 0.02$); whereas no response to Cu level within $CuSO_4$ treatments ($P > 0.28$). Moreover, the interaction was also observed on adjusted piglet weight gain ($P = 0.02$), which showed an increasing linear response to increasing copper level ($P = 0.02$) within TBCC treatments; but a decreasing linear response within $CuSO_4$ treatments ($P = 0.05$).

Table 3.4. Distribution of number of litters for gilts and sows[1,2]

Copper source:	Tribasic copper chloride			Copper sulfate		
Copper level, mg/kg:	20	120	220	20	120	220
Item Diet No.:	1	2	3	4	5	6
No. of gilts allotted	5	6	5	5	5	5
No. of gilts did not farrow	1	0	0	0	0	0
No. of sows that farrowed						
1 Litter	1	0	0	0	0	0
2 Litters	0	0	0	1	1	1
3 Litters	0	2	1	0	2	0
4 Litters	3	4	4	4	2	4
Total	4	6	5	5	5	5
No. of litters						
1st-Parity litters	4	6	5	5	5	5
2nd-Parity litters	4	6	5	5	5	5
3rd-Parity litters	3	6	5	4	4	4
4th-Parity litters	3	4	4	4	2	4
Total	14	22	19	18	16	18
Average No. of litters per sow	3.5	3.7	3.8	3.6	3.2	3.6

[1]No significant difference across treatments.

[2]One gilt (ID:1804) from treatment 1 did not farrow throughout the experiment, and one sow (ID: 1877) from treatment 3 died after completion of the third parity.

Table 3.5. Effects of dietary copper source and level on sow performance[1]

Copper source:	Tribasic copper chloride			Copper sulfate					
Copper level, mg/kg:	20	120	220	20	120	220		*P* values	
Item Diet No.:	1	2	3	4	5	6	SEM	Source	Level
No. of litters	8	15	12	13	11	8			
Sow weight, kg									
Breeding[2]	208.7	206.7	213.6	204.9	207.3	205.3	6.2	0.45	0.90
Late gestation	235.2	237.4	245.0	231.4	232.6	234.5	6.8	0.26	0.64
Post farrowing	215.9	223.0	228.6	214.6	216.7	219.4	6.6	0.31	0.47
Weaning	232.0	232.7	242.4	226.9	235.5	228.1	5.8	0.26	0.62
Sow weight changes, kg									
Breeding to late gestation[2]	33.3	30.7	31.2	28.3	27.9	35.5	4.7	0.77	0.68
Late gestation to post farrowing	-21.1	-16.5	-18.6	-18.8	-17.9	-17.0	2.8	0.72	0.61
Post farrowing to weaning[2]	9.9	6.7	10.9	9.4	11.7	5.7	3.4	0.93	0.93
Late gestation to weaning[2]	-11.0	-9.7	-7.6	-9.4	-6.3	-11.4	4.7	0.92	0.89
Lactation daily feed intake, kg/d[3]	5.78	5.82	6.42	5.82	5.98	5.44	0.20	0.13	0.80

[1]Data were collected from the second- to fourth-parity sows.
[2]Sow parity effect ($P < 0.10$).
[3]Copper source × copper level interaction ($P = 0.02$). A linear response to increasing copper level ($P = 0.03$) within tribasic copper chloride treatments whereas a quadratic response within copper sulfate treatments ($P = 0.06$).

Table 3.6. Effects of dietary copper source and level on litter performance[1]

Copper source:	Tribasic copper chloride			Copper sulfate					
Copper level, mg/kg:	20	120	220	20	120	220		*P* values	
Item / Diet No.:	1	2	3	4	5	6	SEM	Source	Level
No. of litters	8	15	12	13	11	8			
Litter size									
Total born	10.67	10.64	11.51	10.93	10.02	9.83	0.83	0.33	0.83
Live born	10.00	9.77	10.36	10.47	9.77	9.61	0.83	0.89	0.85
Stillborn	0.56	0.81	1.16	0.47	0.33	0.22	0.26	0.03	0.81
Weaning	9.11	8.58	9.09	9.17	8.45	8.28	0.70	0.61	0.66
Mortality	0.22	0.52	0.60	0.63	0.65	0.78	0.28	0.31	0.69
Survival rate, %[6]	97.38	95.25	94.53	95.05	92.91	91.72	2.47	0.23	0.49
Litter weight, kg									
Total born	17.12	15.96	18.52	14.86	15.57	16.34	1.20	0.11	0.35
Live born	16.26	14.84	16.77	14.32	15.25	15.54	1.25	0.37	0.66
Weaning	58.38	55.30	58.68	57.02	53.02	47.66	4.52	0.19	0.59
Litter gain[6]	43.94	42.01	44.10	44.20	39.45	34.25	3.54	0.17	0.40
Piglet weight, kg									
Total born[5]	1.53	1.54	1.67	1.41	1.56	1.59	0.10	0.46	0.30
Live born[2]	1.54	1.56	1.74	1.42	1.57	1.61	0.10	0.31	0.17
Weaning	6.40	6.48	7.10	6.35	6.41	5.88	0.35	0.13	0.95
Piglet gain[4, 6]	4.82	4.92	5.33	4.91	4.80	4.24	0.29	0.12	0.95

Continued

Table 3.6 continued

Adjusted weight, kg									
Litter weight at weaning	57.80	56.59	61.14	57.52	53.68	49.01	4.17	0.14	0.79
Piglet weight at weaning[3]	6.34	6.65	7.41	6.44	6.49	6.06	0.31	0.07	0.57
Litter weight gain[6]	43.36	43.31	46.56	44.70	40.11	35.60	3.14	0.10	0.63
Piglet weight gain[4, 6]	4.76	5.08	5.64	5.00	4.88	4.41	0.24	0.05	0.84

[1]Data were collected from the second- to fourth-parity litters.

[2]Linear response to copper level ($P = 0.06$).

[3]Copper source × copper level interaction ($P = 0.06$). A linear response to increasing copper level ($P = 0.02$) within tribasic copper chloride treatments whereas no response within copper sulfate treatments ($P > 0.28$).

[4]Copper source × copper level interaction ($P = 0.02$). An increasing linear response to increasing copper level ($P = 0.02$) within tribasic copper chloride treatments whereas a decreasing linear response within copper sulfate treatments ($P = 0.05$).

[5]Tendency of linear response to copper level ($P = 0.12$).

[6]Because 1 piglet was sacrificed at birth for the third and fourth litters, adjustment of excluding the sacrificed piglets was applied on live born litter size and litter weight that was used in the calculations of survival rate, litter gain, and piglet gain.

3.4.2 Apparent total tract digestibility

During late gestation (d 98 to 102 of gestation, Table 3.7), sows fed the TBCC diets had lower ATTD of EE ($P = 0.01$) and Fe ($P = 0.08$) than those fed with $CuSO_4$ diets. The ATTD of Cu ($P < 0.0001$) and Fe ($P = 0.06$) linearly increased with increasing dietary Cu levels. There was an interaction of Cu source and level on the ATTD of Cu ($P = 0.0001$). The ATTD of Cu decreased from 20 to 120 mg/kg diets, and then increased from 120 to 220 mg/kg diets within TBCC treatments, whereas it increased from 20 to 120 mg/kg diets and then plateaued thereafter within $CuSO_4$ treatments (linear and quadratic, $P < 0.05$). Sow parity affected the ATTD of EE and Fe ($P < 0.05$), which showed a lower ATTD of EE, but higher ATTD of Fe for sows in their second parity than the third parity. However, because of the lack of interaction between parity and Cu source or level, it is assumed that the parity effects were unbiased across treatments.

Regarding lactation (d 15 to 17 of lactation, Table 3.8), sows fed TBCC diets had greater ATTD of DM, nitrogen, and P ($P < 0.05$), as well as GE and Ca ($P < 0.10$); but lower ATTD of Mn ($P = 0.01$), when compared to the ones fed $CuSO_4$ diets. The increasing dietary Cu levels elevated the ATTD of DM and Cu (linear, $P < 0.05$), Mn (quadratic, $P = 0.02$), and GE (linear, $P = 0.07$). Moreover, sows had greater ATTD of nitrogen and Zn in the second parity than in the third parity.

Sows fed TBCC diets were estimated to excrete less Cu ($P = 0.03$) and Ca ($P = 0.08$) per day during late gestation; and less nitrogen and Fe ($P < 0.05$), as well as P and Cu ($P < 0.10$) during lactation, when compared to sows fed $CuSO_4$ diets (Table 3.9). The estimated fecal excretion of Cu linearly increased ($P < 0.05$), whereas that of Mn decreased (linear and quadratic, $P < 0.05$) as dietary Cu levels increased in the periods of both late

gestation and lactation. The increasing Cu levels did not affect estimated Zn excretion during late gestation ($P = 0.72$) but decreased the amount of excreted Zn in lactation (linear, $P = 0.003$). Estimated fecal excretion of nitrogen, Ca, and P was not affected by increasing dietary Cu levels during late gestation and lactation ($P > 0.48$).

Table 3.7. Effects of dietary copper source and level on apparent total tract digestibility (%) during late gestation[1]

Copper source:	Tribasic copper chloride			Copper sulfate					
Copper level, mg/kg:	20	120	220	20	120	220		*P* values	
Item Diet No.:	1	2	3	4	5	6	SEM	Source	Level
No. of observations	6	11	10	9	7	9			
Dry matter	88.30	87.89	87.55	87.61	88.03	88.32	0.57	0.88	0.99
Nitrogen	86.66	86.48	85.30	86.72	86.34	86.99	0.73	0.38	0.77
Gross energy	88.57	88.13	87.79	87.88	88.43	88.66	0.57	0.73	0.99
Ether extract[3]	86.50	85.71	88.72	91.66	89.40	89.80	1.40	0.01	0.40
Minerals									
Calcium	32.73	27.51	30.21	27.15	28.98	31.86	5.04	0.84	0.83
Phosphorous	34.48	31.04	34.52	32.94	33.83	35.81	3.36	0.75	0.69
Copper[2, 4]	3.66	-4.34	18.04	-15.90	24.59	22.15	4.84	0.27	< 0.0001
Iron[3, 5]	4.14	-6.13	-8.41	0.98	3.47	0.66	4.23	0.08	0.06
Manganese	-5.85	-8.17	6.99	3.09	2.97	11.18	5.39	0.14	0.33
Zinc	5.67	0.75	0.15	7.96	0.89	0.86	5.24	0.81	0.43

[1]Fecal samples were collected from the second- and third-parity sows during d 98 to 102 of gestation.
[2]Copper source × copper level interaction ($P = 0.0001$). The ATTD of Cu decreased from 20 to 120 mg/kg diets, and then increased from 120 to 220 mg/kg diets within TBCC treatments, whereas increased from 20 to 120 mg/kg diets and then plateaued thereafter within $CuSO_4$ treatments (linear and quadratic, $P < 0.05$).
[3]Sow parity effect ($P < 0.05$).
[4]Linear response to copper level ($P < 0.05$).
[5]Tendency of linear response to copper level ($P < 0.10$).

Table 3.8. Effects of dietary copper source and level on apparent total tract digestibility (%) during lactation[1]

Copper source:	Tribasic copper chloride			Copper sulfate					
Copper level, mg/kg:	20	120	220	20	120	220		*P* values	
Item Diet No.:	1	2	3	4	5	6	SEM	Source	Level
No. of observations	6	12	10	9	7	6			
Dry matter[3]	87.17	88.15	87.51	86.41	86.79	87.65	0.36	0.03	0.09
Nitrogen[2]	87.90	89.25	88.16	87.22	87.36	87.96	0.54	0.04	0.38
Gross energy[5]	87.71	88.33	87.82	86.70	87.04	88.16	0.41	0.06	0.19
Ether extract	76.61	78.51	73.78	67.23	76.04	79.70	3.57	0.51	0.28
Minerals									
Calcium	43.52	44.88	43.90	40.57	43.72	35.73	2.65	0.07	0.24
Phosphorous[6]	44.57	47.28	48.07	44.57	45.80	40.17	1.97	0.04	0.33
Copper[3, 6]	16.91	16.58	18.65	9.55	12.34	26.74	3.56	0.69	0.03
Iron	-1.41	-3.41	-7.93	-4.38	-9.27	-2.90	4.12	0.71	0.70
Manganese[4, 5]	4.87	9.30	7.42	7.18	16.42	13.31	2.17	0.01	0.01
Zinc[2]	8.36	12.94	15.46	8.74	11.15	11.18	2.92	0.44	0.28

[1]Fecal samples were collected from the second- and third-parity sows during d 15 to 17 of gestation.
[2]Sow parity effect ($P < 0.05$).
[3]Linear response to copper level ($P = 0.02$).
[4]Quadratic response to copper level ($P = 0.01$).
[5]Tendency of linear response to copper level ($P < 0.06$).
[6]Tendency of copper source × copper level interaction ($P < 0.09$).

Table 3.9. Effects of dietary copper source and level on predicted fecal excretion of nutrients during late gestation and lactation[1]

Copper source:	Tribasic copper chloride			Copper sulfate					
Copper level, mg/kg:	20	120	220	20	120	220		*P* values	
Item Diet No.:	1	2	3	4	5	6	SEM	Source	Level
Late gestation									
No. of observations	6	11	10	9	7	9			
Nitrogen, g/d	6.37	6.26	6.70	6.63	6.52	6.28	0.35	0.91	0.94
Calcium, g/d	8.51	8.92	9.52	10.40	9.72	9.38	0.57	0.08	0.96
Phosphorous, g/d	8.45	8.34	8.50	8.92	8.74	8.71	0.41	0.29	0.94
Trace mineral, mg/d									
Copper[2]	61.3	200.8	346.2	84.8	232.4	361.9	12.8	0.03	< 0.0001
Iron	669.7	641.4	654.3	687.7	706.6	708.3	33.2	0.10	0.97
Manganese[2]	184.9	158.6	154.6	162.7	160.4	162.1	6.3	0.41	0.04
Zinc	326.3	312.9	320.5	303.2	300.2	316.4	15.2	0.29	0.72
Lactation									
No. of observations	6	12	10	9	7	6			
Nitrogen, g/d	20.63	18.70	21.98	23.95	23.68	19.92	1.13	0.03	0.48
Calcium, g/d	22.80	21.76	24.20	24.28	24.42	24.18	1.31	0.21	0.70
Phosphorous, g/d	22.42	21.46	23.25	24.08	23.67	23.78	0.91	0.06	0.55
Trace mineral, mg/d									
Copper[2]	128.2	558.7	1127.4	192.9	734.8	1074.2	44.8	0.10	< 0.0001
Iron	2,130	2,040	2,263	2,410	2,483	2,196	96	0.01	0.91

Continued

Table 3.9 continued

Manganese[2, 3]	594.7	434.4	442.0	517.1	490.2	445.1	20.3	0.71	< 0.0001
Zinc[2]	994.2	850.2	874.1	922.9	872.4	809.8	34.8	0.19	0.01

[1]Fecal samples were collected from the second- and third-parity sows during d 98 to 102 of gestation and d 15 to 17 of lactation.
[2]Linear response to copper level ($P < 0.05$).
[3]Quadratic response to copper level ($P < 0.05$).

3.4.3 Hematology and antioxidant status of sows and piglets

With regard to late gestation sows, hematocrit, the activity of serum SOD and Cp, and serum MDA level were not affected by dietary Cu source ($P > 0.31$); however, TBCC fed sows tended to have a greater level of hemoglobin than $CuSO_4$ fed sows ($P = 0.10$; Table 3.10). The activity of total and Cu/Zn SOD tended to increase linearly ($P < 0.10$) as dietary Cu level increased.

During lactation (Table 3.11), sows fed TBCC diets had greater activity of total SOD ($P = 0.08$), Cu/Zn SOD ($P = 0.04$), and Cp ($P = 0.01$) when compared to sows fed the $CuSO_4$ diets. The activity of total and Cu/Zn SOD increased linearly ($P < 0.03$) with increasing dietary Cu levels. In addition, the activity of Mn SOD and Cp quadratically changed with increasing dietary Cu levels ($P < 0.05$).

Regarding neonatal piglets, hemoglobin and serum total SOD activity tended to increase linearly ($P < 0.10$), and Cu/Zn SOD activity tended to increase quadratically ($P = 0.09$), with increasing Cu levels in the sow diets (Table 3.11). However, dietary Cu source did not affect the measurements of neonatal piglets. In contrast, weanling piglets from sows fed with TBCC diets tended to have lower levels of Htc and Hb ($P < 0.10$) than those from sows fed with $CuSO_4$ diets (Table 3.11). Moreover, weanling piglets from sows fed TBCC diets had higher Cp activity in serum when compared to piglets from sows fed $CuSO_4$ diets ($P < 0.05$).

Table 3.12 shows antioxidant status in colostrum and milk samples. Total SOD activity in colostrum samples from sows fed TBCC diets was lower than that from sows that fed $CuSO_4$ diets ($P < 0.05$). In milk samples, total and Cu/Zn SOD activity decreased from 20

to 120 mg/kg diets and then increased from 120 to 220 mg/kg diets for both Cu sources (quadratic, $P < 0.05$).

Sow parity effects were observed on serum SOD activity in gestation and lactating sows, as well as piglet values at weaning (Table 3.10 and 3.11); however, no significant interactions between sow parity and Cu source or level was observed in this experiment, demonstrating that sow parity effects were consistent across dietary treatments.

Table 3.10. Effects of dietary copper source and level on Htc, Hb, and antioxidant status of sows[1, 2]

Copper source:	Tribasic copper chloride			Copper sulfate					
Copper level, mg/kg:	20	120	220	20	120	220		*P* values	
Item Diet No.:	1	2	3	4	5	6	SEM	Source	Level
Late gestation									
No. of observations	6	11	10	9	8	9			
Htc, %[3]	39.88	38.60	38.70	37.54	37.73	38.80	1.24	0.31	0.87
Hb, g/dL	13.56	12.87	12.96	12.39	12.42	12.98	0.39	0.10	0.62
Total SOD, U/mL[3, 6]	47.52	49.85	52.61	48.04	50.52	53.84	3.05	0.75	0.22
Cu/Zn SOD, U/mL[6]	29.59	30.96	34.03	28.81	33.84	34.27	2.86	0.74	0.24
Mn SOD, U/mL[3]	17.93	18.89	18.57	19.23	16.68	19.57	1.60	0.98	0.71
Cp, U/mL	0.113	0.123	0.094	0.104	0.101	0.116	0.017	0.85	0.92
MDA, µM	4.70	4.46	4.79	4.38	4.72	4.95	0.33	0.91	0.56
Lactation									
No. of observations	8	15	13	13	10	12			
Htc, %[3]	35.58	35.40	34.92	33.29	34.95	34.54	1.04	0.23	0.78
Hb, g/dL	11.62	11.92	11.80	11.28	11.53	11.64	0.34	0.30	0.67
Total SOD, U/mL[3, 4]	39.57	43.09	45.77	37.16	39.30	41.74	2.36	0.08	0.09
Cu/Zn SOD, U/mL[3, 4]	24.90	25.58	30.92	21.29	22.73	26.82	2.05	0.04	0.02
Mn SOD, U/mL[3, 5]	14.67	17.51	14.86	15.87	16.57	14.92	1.14	0.91	0.14
Cp, U/mL[5, 6]	0.153	0.164	0.130	0.128	0.139	0.117	0.010	0.01	0.02
MDA, µM	6.69	6.94	6.54	6.11	7.01	6.12	0.48	0.45	0.32

[1]Htc, hematocrit; Hb, hemoglobin; SOD, superoxide dismutase; Cp, ceruloplasmin; MDA, malondialdehyde.

[2]Blood samples were collected on d 100 of gestation from the second- and third-parity sows; and on d 15 of lactation from the second- to fourth-parity sows.
[3]Sow parity effect ($P < 0.05$).
[4]Linear response to copper level ($P < 0.05$).
[5]Quadratic response to copper level ($P < 0.05$).
[6]Tendency of linear response to copper level ($P < 0.10$).

Table 3.11. Effects of dietary copper source and level on Htc, Hb, and antioxidant enzyme activity of piglets[1, 2, 3]

Copper source:	Tribasic copper chloride			Copper sulfate					
Copper level, mg/kg:	20	120	220	20	120	220		*P* values	
Item Diet No.:	1	2	3	4	5	6	SEM	Source	Level
At Birth									
No. of observations	5	11	9	8	6	8			
Htc, %	26.42	28.06	32.14	31.77	26.61	34.17	2.77	0.39	0.09
Hb, g/dL[5]	8.08	8.62	10.21	9.60	8.72	10.42	0.80	0.36	0.08
Total SOD, U/mL[5]	49.07	67.23	66.21	62.35	64.76	65.80	5.49	0.45	0.14
Cu/Zn SOD, U/mL[6]	38.09	46.86	48.37	39.45	49.90	40.25	4.49	0.74	0.14
Mn SOD, U/mL	10.98	20.37	17.83	22.91	14.87	25.55	5.25	0.28	0.61
Cp, U/mL	0.006	0.006	0.004	0.003	0.004	0.004	0.002	0.42	0.78
MDA, µM	9.89	11.68	11.49	15.68	11.12	11.97	1.89	0.23	0.77
At Weaning									
No. of observations	16	27	25	24	20	18			
Htc, %	30.48	32.82	31.30	32.50	32.58	34.47	1.09	0.07	0.40
Hb, g/dL[4]	9.16	10.09	9.07	9.85	9.86	10.46	0.40	0.06	0.50
Total SOD, U/mL[4]	54.13	57.34	59.16	54.25	56.96	56.35	2.92	0.67	0.44
Cu/Zn SOD, U/mL[4]	32.17	33.45	31.71	30.26	32.76	34.24	2.24	0.99	0.65
Mn SOD, U/mL[4]	21.96	23.89	27.46	23.99	24.21	22.11	2.10	0.56	0.70
Cp, U/mL[4]	0.094	0.083	0.109	0.070	0.081	0.080	0.008	0.01	0.21
MDA, µM	15.23	14.03	14.45	13.77	14.20	14.69	1.38	0.57	0.81

[1]Htc, hematocrit; Hb, hemoglobin; SOD, superoxide dismutase; Cp, ceruloplasmin; MDA, malondialdehyde.

[2]Blood samples that collected from piglets at birth were from the third- and fourth-parity litters; and blood samples that collected from piglets at weaning were from the second- to fourth-parity litters.
[3]No significant effect of piglet sex was detected ($P > 0.29$).
[4]Sow parity effect ($P < 0.05$).
[5]Tendency of linear response to copper level ($P < 0.10$).
[6]Tendency of quadratic response to copper level ($P < 0.10$).

Table 3.12. Effects of dietary copper source and level on antioxidant enzyme activity of milk and colostrum[1, 2]

Copper source:	Tribasic copper chloride			Copper sulfate					
Copper level, mg/kg:	20	120	220	20	120	220		*P* values	
Item Diet No.:	1	2	3	4	5	6	SEM	Source	Level
Colostrum									
No. of observations	5	10	8	8	6	8			
Total SOD, U/mL	86.83	87.13	86.48	93.89	90.21	95.31	2.57	0.01	0.65
Cu/Zn SOD, U/mL	52.42	39.73	45.57	51.49	50.79	40.51	5.99	0.74	0.35
Mn SOD, U/mL	34.41	47.40	40.91	42.41	39.43	54.79	6.16	0.37	0.34
Cp, U/mL	0.005	0.006	0.024	0.004	0.006	0.002	0.006	0.13	0.35
MDA, µM	3.92	4.94	4.50	4.72	4.27	5.62	0.57	0.39	0.45
Milk									
No. of observations	5	9	9	8	6	9			
Total SOD, U/mL[3]	65.73	50.21	57.83	52.03	51.53	63.24	4.06	0.49	0.05
Cu/Zn SOD, U/mL[3]	38.92	33.13	38.82	34.39	30.58	44.30	3.11	0.83	0.01
Mn SOD, U/mL	26.80	17.09	19.01	17.64	20.95	18.95	3.04	0.48	0.50
Cp, U/mL	0.001	0.002	0.002	0.002	0.002	0.002	0.001	0.81	0.78
MDA, µM	2.34	2.10	2.32	2.32	2.67	2.15	0.23	0.52	0.81

[1]Htc, hematocrit; Hb, hemoglobin; SOD, superoxide dismutase; Cp, ceruloplasmin; MDA, malondialdehyde.
[2]Colostrum and milk samples were collected from the third- and fourth-parity sows.
[3]Quadratic response to copper level ($P < 0.05$).

3.4.4 Colostrum and milk composition

Colostrum composition (Table 3.13) was not affected by dietary Cu source or level ($P > 0.13$), except for a tendency of decreasing levels of Fe (linear, $P = 0.09$) and Zn (quadratic, $P = 0.08$) as dietary Cu level increased. In contrast, the milk from sows that were fed TBCC diets had greater levels of protein than that from sows that were fed $CuSO_4$ diets ($P = 0.02$, Table 3.14). Furthermore, the TBCC fed sows tended to have greater levels of fat, gross energy, and total solids, but a lower level of lactose in milk than the $CuSO_4$ fed sows ($P < 0.10$). With increasing dietary Cu levels, concentrations of fat and Cu ($P < 0.05$), as well as gross energy and total solids ($P < 0.10$) linearly increased in milk. However, linearly decreased levels of lactose ($P = 0.03$) and Zn ($P = 0.09$) were observed in milk as dietary Cu level increased. In addition, non-fat solids in milk decreased from sows fed the 20 to 120 mg/kg Cu treatments and then increased from the 120 to 220 mg/kg Cu treatments (quadratic, $P = 0.05$).

The amount of milk yield per litter and piglet were not affected by dietary Cu source or level ($P > 0.52$, Table 3.15). Copper output in milk per piglet was estimated to be greater for sows fed with TBCC diets than those fed with $CuSO_4$ diets ($P = 0.03$). Moreover, the increasing dietary Cu levels increased Cu output in milk (linear, $P = 0.0004$). The amount of total SOD activity output in milk decreased from the 20 to 120 mg/kg Cu diets and then increased from the 120 to 220 mg/kg Cu diets for both Cu sources (quadratic, $P = 0.10$). The milk output of Cu/Zn SOD activity displayed the same response to increasing dietary Cu levels as that of total SOD activity (quadratic, $P = 0.06$).

Table 3.13. Effects of dietary copper source and level on nutrient concentrations in colostrum (as-is basis)[1]

Copper source:	Tribasic copper chloride			Copper sulfate					
Copper level, mg/kg:	20	120	220	20	120	220		*P* values	
Item Diet No.:	1	2	3	4	5	6	SEM	Source	Level
No. of observations	5	10	8	8	7	8			
Fat, %	4.72	5.60	6.88	5.13	6.42	5.46	0.76	0.97	0.21
Protein, %	9.88	10.17	9.27	11.58	9.61	11.40	1.70	0.48	0.61
Lactose, %	3.58	3.57	3.82	3.03	3.69	3.47	0.34	0.37	0.44
Gross energy, Mcal/kg	1.32	1.24	1.32	1.27	1.28	1.30	0.09	0.93	0.81
Total solids, %	21.97	21.08	21.67	21.85	21.40	22.28	1.37	0.84	0.71
Non-fat solids, %	14.38	14.60	13.85	15.77	14.14	15.72	1.48	0.49	0.62
Minerals, μg/mL[4]									
Copper	2.14	2.18	2.13	2.32	2.04	2.06	0.30	0.97	0.90
Iron[2]	2.11	1.75	1.53	1.77	1.75	1.54	0.23	0.56	0.23
Zinc[3]	8.85	6.51	6.41	7.43	6.49	8.07	0.77	0.90	0.13

[1]Colostrum samples were collected from the third- and fourth-parity sows.
[2]Tendency of linear response to copper level ($P = 0.09$).
[3]Tendency of quadratic response to copper level ($P = 0.08$).
[4]Manganese was below the detectable level with atomic absorption spectrophotometer.

Table 3.14. Effects of dietary copper source and level on nutrient concentrations in milk (as-is basis)[1]

Copper source:	Tribasic copper chloride			Copper sulfate					
Copper level, mg/kg:	20	120	220	20	120	220		*P* values	
Item Diet No.:	1	2	3	4	5	6	SEM	Source	Level
No. of observations	5	9	9	8	6	8			
Fat, %[2]	5.72	5.78	6.19	4.98	5.06	6.03	0.38	0.10	0.07
Protein, %	4.60	4.62	4.75	4.44	4.19	4.57	0.12	0.02	0.11
Lactose, %[2]	5.95	5.95	5.93	6.16	6.00	5.93	0.06	0.10	0.10
Gross energy, Mcal/kg[4]	1.03	1.03	1.08	0.96	0.94	1.05	0.04	0.07	0.06
Total solids, %[4]	17.26	17.31	17.93	16.50	16.22	17.57	0.45	0.06	0.05
Non-fat solids, %[3]	10.97	10.90	11.08	10.95	10.61	10.90	0.12	0.11	0.13
Minerals, µg/mL[5]									
Copper[2]	1.12	1.23	1.34	1.01	1.12	1.36	0.05	0.13	< 0.0001
Iron	2.05	1.66	1.51	1.38	1.63	1.48	0.23	0.22	0.62
Zinc[2]	4.41	3.83	3.74	4.20	3.33	3.49	0.39	0.32	0.17

[1]Milk samples were collected from the third- and fourth-parity sows.
[2]Linear response to copper level ($P < 0.05$).
[3]Quadratic response to copper level ($P < 0.05$).
[4]Tendency of linear response to copper level ($P < 0.10$).
[5]Manganese was below the detectable level with atomic absorption spectrophotometer.

Table 3.15. Effects of dietary copper source and level on predicted enzyme and nutrient outputs in milk during lactation

Copper source:	Tribasic copper chloride			Copper sulfate					
Copper level, mg/kg:	20	120	220	20	120	220		*P* values	
Item Diet No.:	1	2	3	4	5	6	SEM	Source	Level
No. of observations	4	9	8	8	6	5			
Milk yield per litter, L[1]	176.4	162.3	170.1	180.5	161.7	160.3	14.7	0.87	0.55
Milk yield per piglet, L[2]	18.44	19.61	19.69	19.18	19.10	18.63	0.71	0.65	0.77
Enzyme and nutrient outputs in milk during lactation, per piglet[3]									
Total SOD, million U[5]	1.12	1.00	1.08	0.99	0.98	1.16	0.07	0.72	0.13
Cu/Zn SOD, million U[5]	0.70	0.64	0.72	0.65	0.59	0.82	0.07	0.94	0.06
Mn SOD, million U	0.42	0.36	0.37	0.34	0.40	0.34	0.05	0.56	0.81
Copper, mg[4]	23.01	23.69	26.60	19.03	21.46	26.22	1.16	0.03	0.0004
Iron, mg	32.55	34.09	28.39	26.32	30.65	29.31	3.90	0.38	0.61
Zinc, mg[5]	85.49	73.12	77.02	80.19	63.99	73.48	7.35	0.34	0.19

[1]Milk yield per litter was predicted by a Bayesian hierarchical model based on litter size and litter weight gain (Hansen et al., 2012). The density of milk was predicted by an equation that included temperature (°C), milk fat content (%), milk protein content (%), and lactose and other solids (%) (Ueda, 1999).

[2]The average of litter size born alive and at weaning alive was used to estimate milk yield per piglet.

[3]Nutrient concentrations in milk samples from d 15 to 17 of lactation were used to estimate their outputs during the lactation period.

[4]Linear response to copper level ($P < 0.05$).

[5]Tendency of quadratic response to copper level ($P < 0.10$).

3.4.5 Organ weights and tissue trace mineral levels

The absolute and relative organ weights of sows were not affected by dietary Cu source or level ($P > 0.20$, Table 3.16), except for greater absolute ($P = 0.09$) and relative ($P = 0.02$) liver weight for sows fed with $CuSO_4$ compared to those fed with TBCC. Moreover, the experimental sows had greater slaughter weight, absolute weight of liver, heart, and kidney, as well as relative weight of liver and kidney than the baseline gilts ($P < 0.05$). The absolute and relative organ weights of piglets at birth and weaning were not affected by dietary Cu source or level ($P > 0.15$, Table 3.17 and 3.18).

The experimental sows had a greater concentration of Cu (liver and heart), Fe (heart), Mn (liver), and Zn (liver, kidney, and heart) when compared to trace mineral concentrations in various organs of baseline gilts ($P < 0.05$). Moreover, the experimental sows tended to have greater concentrations of Cu and Fe in the kidney as well as Fe in ovary than the baseline gilts ($P < 0.10$). Sows fed with the TBCC diets had lower concentrations of Cu ($P = 0.04$), but greater concentrations of Fe and Mn ($P < 0.05$) in liver than sows fed the $CuSO_4$ diets (Table 3.19). In addition, liver Cu concentration increased with increasing dietary Cu levels (linear and quadratic, $P < 0.05$). Trace mineral concentrations of kidney, heart, and ovary were not affected by either Cu source or level ($P > 0.11$). A Cu source × Cu level interaction ($P = 0.04$) was detected in kidney Mn concentrations. Manganese concentration did not respond to increasing dietary Cu levels ($P > 0.31$) within the TBCC treatments; whereas it was linearly increased as dietary copper level increased ($P = 0.04$) within $CuSO_4$ treatments.

Tissue trace mineral contents are presented in Table 3.20. The experimental sows had greater contents of Cu (liver and heart), Fe (kidney and heart), Mn (liver), and Zn (liver,

kidney, and heart) than those of baseline gilts ($P < 0.05$). In addition, Cu and Mn contents in the kidney tended to be greater in experimental sows than in baseline gilts ($P < 0.10$). Sows fed the TBCC diets had lower liver Cu content ($P = 0.01$) but greater liver Fe content ($P = 0.10$) when compared to sows fed the $CuSO_4$ diets. The increasing dietary Cu levels increased liver Cu content (linear, $P < 0.0001$) and heart Zn content (quadratic, $P = 0.04$).

Trace mineral concentrations and contents in various organs of neonatal piglets were not affected by Cu source or level of the sow diets ($P > 0.16$, Table 3.21 and 3.22), except that heart Zn content in piglets from TBCC fed sows was greater ($P = 0.05$) than that in piglets from $CuSO_4$ fed sows. In addition, kidney Cu concentration tended to increase (quadratic, $P = 0.08$) as dietary Cu level increased.

Regarding trace mineral concentrations and contents in tissues of weanling piglets (Table 3.23 and 3.24), weanling piglets from sows fed the TBCC diets had lower Fe concentration and content in kidney than those from sows fed the $CuSO_4$ diets ($P < 0.05$). In addition, increasing Cu levels of sow diets resulted in an increase of Cu concentration and total content in the liver of weanling piglets (linear, $P < 0.01$). However, Zn concentrations and contents in liver tended to decrease as maternal dietary Cu levels increased (linear, $P < 0.10$).

There was no effect of sow parity and piglet sex on trace mineral concentrations or contents of various organs in piglets at birth. A sow parity effect was detected on Cu and Zn concentrations in the heart of weanling pigs ($P < 0.01$). However, since no significant interaction was detected between sow parity and Cu source, or between sow parity and Cu level, the sow parity effects were unbiased across treatments.

Table 3.16. Effects of dietary copper source and level on organ weights of gilts and sows[1, 2]

Copper source:	Tribasic copper chloride			Copper sulfate						
Copper level, mg/kg:	20	120	220	20	120	220	Baseline		*P* values	
Item Diet No.:	1	2	3	4	5	6	gilts	SEM	Source	Level
No. of observations	3	6	4	4	4	4	6			
Slaughter weight, kg[3]	212.7	209.3	212.5	210.3	197.3	206.3	182.2	7.5	0.31	0.62
Organ absolute weight, g										
Liver[3]	2186	2148	2113	2198	2317	2556	1682	118	0.09	0.27
Heart[3]	751	705	727	689	761	800	577	48	0.61	0.73
Kidney[3]	522	525	493	545	530	479	353	51	0.82	0.38
Ovary	19	23	17	16	18	20	18	2	0.20	0.66
Organ relative weight, %[5]										
Liver[3]	1.04	1.03	1.00	1.05	1.18	1.24	0.96	0.06	0.02	0.49
Heart	0.353	0.336	0.345	0.329	0.387	0.388	0.331	0.022	0.24	0.58
Kidney[4]	0.247	0.252	0.233	0.261	0.271	0.233	0.202	0.027	0.71	0.44
Ovary	0.009	0.011	0.008	0.007	0.009	0.010	0.010	0.001	0.31	0.28

[1]A single degree of freedom contrast was conducted to compare experimental sows vs. baseline gilts.
[2]Data were collected from sows that completed the third- and fourth-parity.
[3]Difference between experimental sows vs. baseline gilts ($P < 0.05$).
[4]Tendency of difference between experimental sows vs. baseline gilts ($P < 0.10$).
[5]As a percentage of slaughter weight.

Table 3.17. Effects of dietary copper source and level on organ weights of piglets at birth[1]

Copper source:	Tribasic copper chloride			Copper sulfate					
Copper level, mg/kg:	20	120	220	20	120	220		*P* values	
Item Diet	1	2	3	4	5	6	SEM	Source	Level
No. of observations	5	10	9	8	6	8			
Slaughter weight, kg	1.65	1.51	1.62	1.35	1.55	1.50	0.07	0.05	0.62
Organ absolute weight, g									
Liver	43.20	39.29	40.61	39.20	42.81	38.50	3.50	0.88	0.94
Kidney	10.64	10.80	11.85	9.58	10.70	10.69	0.85	0.37	0.29
Heart	10.30	9.47	11.04	8.73	10.39	9.27	0.62	0.15	0.57
Organ relative weight, %[2]									
Liver	2.58	2.61	2.48	2.89	2.77	2.55	0.16	0.15	0.44
Kidney	0.643	0.712	0.728	0.710	0.693	0.714	0.039	0.56	0.37
Heart	0.626	0.626	0.682	0.649	0.672	0.620	0.026	0.93	0.87

[2]Data were collected from sows that completed the third- and fourth-parity.
[2]As a percentage of slaughter weight.

Table 3.18. Effects of dietary copper source and level on organ weights of piglets at weaning[1]

Copper source:	Tribasic copper chloride			Copper sulfate					
Copper level, mg/kg:	20	120	220	20	120	220		*P* values	
Item Diet No.:	1	2	3	4	5	6	SEM	Source	Level
No. of observations	4	9	10	8	5	8			
Slaughter weight, kg	6.17	6.55	7.14	6.32	6.35	5.46	0.46	0.13	0.90
Organ absolute weight, g									
Liver	163.23	180.73	178.51	172.60	166.95	154.06	15.30	0.45	0.86
Kidney	34.00	37.76	38.75	35.79	36.11	33.63	3.03	0.51	0.83
Heart	35.02	36.19	41.23	37.51	37.55	32.53	3.74	0.61	0.99
Organ relative weight, %[2]									
Liver	2.66	2.76	2.52	2.69	2.61	2.80	0.10	0.52	0.95
Kidney	0.553	0.574	0.548	0.566	0.569	0.613	0.025	0.24	0.70
Heart	0.569	0.547	0.588	0.580	0.591	0.584	0	0.50	0.83

[2]Data were collected from sows that completed the third- and fourth-parity.
[2]As a percentage of slaughter weight.

Table 3.19. Effects of dietary copper source and level (mg/kg) on tissue trace mineral concentration (DM basis, mg/kg) of sows[1, 2]

Copper source:	Tribasic copper chloride			Copper sulfate						
Copper level:	20	120	220	20	120	220	Baseline		*P* values	
Item Diet No.:	1	2	3	4	5	6	gilts	SEM	Source	Level
No. of observations	3	6	4	4	4	4	6			
Liver										
Copper[3, 4, 5]	123.9	232.5	923.0	298.0	459.8	1372.5	72.1	129.8	0.04	< 0.0001
Iron	1363.9	1416.6	979.6	934.0	993.9	852.1	1156.1	159.5	0.04	0.41
Manganese[3]	8.29	8.07	7.03	6.82	6.84	6.73	6.12	0.58	0.04	0.14
Zinc[3]	260.1	234.1	226.4	255.0	236.3	282.9	165.0	27.1	0.46	0.58
Kidney										
Copper[7]	46.62	38.89	40.73	39.51	36.91	52.71	23.14	11.36	0.97	0.98
Iron[7]	290.4	260.9	269.2	230.4	266.4	231.9	211.1	28.7	0.26	0.76
Manganese[6]	6.57	5.30	5.60	4.25	6.49	6.36	5.56	0.58	0.85	0.59
Zinc[3]	118.4	101.1	114.4	92.2	111.2	117.6	89.5	10.1	0.62	0.74
Heart										
Copper[3]	11.51	11.47	11.91	11.58	12.10	14.05	9.82	1.06	0.37	0.43
Iron[3]	185.5	194.6	183.3	183.0	195.7	186.2	168.4	8.5	0.93	0.37
Manganese	1.80	1.59	1.76	1.69	1.57	2.53	1.74	0.37	0.56	0.40
Zinc[3]	58.61	57.14	59.88	55.99	59.96	67.35	51.46	4.13	0.52	0.32
Ovary										
Copper	9.88	7.62	10.15	7.69	7.27	8.56	5.34	2.18	0.47	0.99
Iron[7]	215.1	330.8	387.5	291.5	260.0	255.9	396.7	59.7	0.44	0.54
Manganese	2.24	2.16	2.28	2.00	1.89	2.08	2.20	0.19	0.11	0.91
Zinc	38.14	38.47	41.37	40.62	42.66	41.55	41.88	1.74	0.13	0.48

[1]A single degree of freedom contrast was conducted to compare experimental sows vs. baseline gilts.

[2]Tissue samples were collected from sows that completed the third- and fourth-parity.
[3]Difference for the contrast of experimental sows vs. baseline gilts ($P < 0.05$).
[4]Linear response to copper level ($P < 0.0001$).
[5]Quadratic response to copper level ($P = 0.04$).
[6]Copper source × copper level interaction ($P = 0.04$). Manganese concentration did not respond to increasing dietary Cu level ($P > 0.31$) within tribasic copper chloride treatments; whereas linearly increased as dietary copper level increased ($P = 0.04$) within copper sulfate treatments.
[7]Tendency of difference between experimental sows vs. baseline gilts ($P < 0.10$).

Table 3.20. Effects of dietary copper source and level (mg/kg) on total content of tissue trace mineral (mg) of sows[1, 2]

Copper source:	Tribasic copper chloride			Copper sulfate						
Copper level:	20	120	220	20	120	220	Baseline		*P* values	
Item Diet No.:	1	2	3	4	5	6	gilts	SEM	Source	Level
No. of	3	6	4	4	4	4	6			
Liver										
Copper[3, 4]	77.77	136.86	569.24	185.78	320.33	970.71	37.26	85.31	0.01	< 0.0001
Iron	867.1	890.8	626.5	578.7	681.8	626.3	588.7	101.3	0.10	0.73
Manganese[3]	5.49	4.98	4.50	4.37	4.71	4.68	3.10	0.43	0.29	0.47
Zinc[3]	173.13	144.09	145.00	160.66	162.84	201.38	83.76	20.34	0.26	0.83
Kidney										
Copper[6]	4.45	4.17	3.66	4.41	3.71	5.07	1.77	1.36	0.85	0.90
Iron[3]	26.30	24.35	25.43	23.44	25.61	21.73	15.84	2.38	0.45	0.91
Manganese[6]	0.607	0.518	0.520	0.455	0.640	0.603	0.418	0.080	0.82	0.90
Zinc[3]	10.98	10.13	10.46	9.75	10.95	10.98	6.73	1.41	0.98	0.77
Heart										
Copper[3]	2.28	1.81	2.06	1.77	1.93	2.54	1.36	0.18	0.90	0.10
Iron[3]	36.60	30.77	31.61	27.94	31.10	34.48	23.23	2.30	0.40	0.65
Manganese	0.357	0.246	0.300	0.258	0.253	0.450	0.242	0.058	0.74	0.18
Zinc[3, 5]	11.57	9.00	10.35	8.54	9.52	12.31	7.10	0.78	0.78	0.05
Ovary										
Copper	0.043	0.032	0.035	0.030	0.025	0.030	0.017	0.011	0.39	0.88
Iron	0.773	1.347	1.145	1.513	0.833	0.933	1.227	0.310	0.97	0.93
Manganese	0.008	0.009	0.007	0.010	0.006	0.008	0.007	0.002	0.81	0.71
Zinc	0.140	0.162	0.128	0.205	0.138	0.148	0.138	0.036	0.56	0.71

[1]A single degree of freedom contrast was conducted to compare experimental sows vs. baseline gilts.

[2]Tissue samples were collected from sows that completed the third- and fourth-parity.
[3]Difference for the contrast of experimental sows vs. baseline gilts ($P < 0.05$).
[4]Linear response to copper level ($P < 0.05$).
[5]Quadratic response to copper level ($P < 0.05$).
[6]Tendency of difference between experimental sows vs. baseline gilts ($P < 0.10$).

Table 3.21. Effects of dietary copper source and level on tissue trace mineral concentration (DM basis, mg/kg) of piglets at birth[1]

Copper source:	Tribasic copper chloride			Copper sulfate					
Copper level, mg/kg:	20	120	220	20	120	220		*P* values	
Item Diet No.:	1	2	3	4	5	6	SEM	Source	Level
No. of observations	5	10	9	8	6	8			
Liver									
Copper	156.8	174.4	182.2	154.2	147.4	188.7	20.5	0.65	0.29
Iron	496.3	793.7	698.7	675.7	571.4	778.1	144.7	0.92	0.58
Manganese	5.62	5.75	6.05	5.14	6.12	6.07	0.43	0.93	0.27
Zinc	357.4	412.7	431.8	406.8	390.4	409.9	61.9	0.97	0.82
Kidney									
Copper[2]	11.85	10.69	11.30	12.36	10.15	13.57	1.18	0.45	0.20
Iron	159.4	150.5	156.6	183.1	155.1	180.0	25.0	0.41	0.74
Manganese	3.68	3.94	3.65	3.91	3.45	3.84	0.32	0.92	0.96
Zinc	67.73	64.88	65.34	66.05	65.23	66.38	1.48	0.94	0.50
Heart									
Copper	10.17	9.92	9.93	10.04	9.96	10.14	0.19	0.80	0.71
Iron	124.3	146.6	143.0	141.9	119.4	141.6	10.9	0.69	0.60
Manganese	2.09	1.96	1.99	2.34	1.86	2.16	0.16	0.44	0.22
Zinc	74.70	73.79	74.47	74.58	71.36	74.04	1.36	0.38	0.31

[1]Tissue samples were collected from piglets in the third- and fourth-parity litters.
[2]Tendency of quadratic response to copper level ($P = 0.08$).

Table 3.22. Effects of dietary copper source and level on total content of tissue trace mineral (mg) of piglets at birth[1]

Copper source:	Tribasic copper chloride			Copper sulfate					
Copper level, mg/kg:	20	120	220	20	120	220		*P* values	
Item Diet No.:	1	2	3	4	5	6	SEM	Source	Level
No. of observations	5	10	9	8	6	8			
Liver									
Copper	1.64	1.80	1.91	1.57	1.59	1.80	0.23	0.49	0.55
Iron	5.00	7.99	6.68	6.49	5.76	7.37	1.10	0.98	0.48
Manganese	0.066	0.058	0.064	0.052	0.067	0.060	0.007	0.68	0.88
Zinc	3.58	4.21	4.57	4.04	4.14	3.79	0.61	0.80	0.80
Kidney									
Copper	0.024	0.023	0.026	0.022	0.022	0.028	0.003	0.98	0.21
Iron	0.322	0.313	0.369	0.330	0.335	0.391	0.069	0.76	0.64
Manganese	0.008	0.008	0.008	0.007	0.008	0.008	0.001	0.46	0.62
Zinc	0.138	0.136	0.151	0.119	0.142	0.136	0.011	0.31	0.36
Heart									
Copper	0.021	0.019	0.022	0.017	0.022	0.019	0.001	0.16	0.65
Iron	0.260	0.285	0.319	0.245	0.253	0.268	0.029	0.76	0.64
Manganese[2]	0.004	0.004	0.004	0.004	0.004	0.004	0.0004	0.63	0.58
Zinc	0.157	0.145	0.167	0.130	0.154	0.138	0.009	0.05	0.62

[1]Tissue samples were collected from piglets in the third- and fourth-parity litters.
[2]Tendency of copper source × copper level interaction ($P = 0.07$).

Table 3.23. Effects of dietary copper source and level on tissue trace mineral concentration (DM basis, mg/kg) of piglets at weaning[1]

Copper source:	Tribasic copper chloride			Copper sulfate					
Copper level, mg/kg:	20	120	220	20	120	220		*P* values	
Item Diet No.:	1	2	3	4	5	6	SEM	Source	Level
No. of observations	5	9	10	8	5	8			
Liver									
Copper[2]	358.1	399.3	537.1	379.7	444.9	541.6	27.0	0.29	< 0.0001
Iron	152.3	343.3	224.1	299.0	207.6	417.5	80.4	0.32	0.46
Manganese	9.69	9.18	10.41	10.55	9.45	9.83	0.67	0.74	0.41
Zinc[3]	391.8	280.3	322.3	384.4	356.3	292.1	42.7	0.72	0.15
Kidney									
Copper	29.86	22.06	29.00	32.47	28.45	32.77	4.57	0.27	0.38
Iron	116.4	171.1	135.1	182.2	175.7	222.7	22.1	0.01	0.38
Manganese	5.03	5.13	5.39	5.45	5.51	4.96	0.41	0.71	0.94
Zinc	89.39	78.71	84.71	88.08	86.28	86.32	3.36	0.35	0.23
Heart									
Copper[4]	8.68	9.06	9.04	8.78	9.17	8.98	0.22	0.79	0.25
Iron	132.2	155.3	139.8	153.4	149.1	173.5	12.3	0.12	0.52
Manganese	1.33	1.38	1.51	1.67	1.06	1.56	0.19	0.90	0.22
Zinc[4]	71.13	67.05	69.73	68.19	66.98	67.77	1.60	0.22	0.30

[1]Tissue samples were collected from piglets in the third- and fourth-parity litters.
[2]Linear response to copper level ($P < 0.0001$).
[3]Tendency of linear response to copper level ($P = 0.06$).
[4]Sow parity effects ($P < 0.05$).

Table 3.24. Effects of dietary copper source and level on total content of tissue trace mineral (mg) of piglets at weaning[1]

Copper source:	Tribasic copper chloride			Copper sulfate					
Copper level, mg/kg:	20	120	220	20	120	220		*P* values	
Item Diet No.:	1	2	3	4	5	6	SEM	Source	Level
No. of observations	5	9	10	8	5	8			
Liver									
Copper[2]	13.70	16.83	23.53	16.43	18.21	19.08	2.05	0.95	0.01
Iron	5.96	13.48	9.78	11.68	8.37	14.22	2.75	0.47	0.47
Manganese	0.367	0.390	0.455	0.437	0.387	0.348	0.038	0.68	0.92
Zinc[3]	15.13	11.69	13.90	16.28	14.78	10.25	2.05	0.91	0.21
Kidney									
Copper	0.184	0.156	0.215	0.219	0.190	0.211	0.038	0.48	0.56
Iron	0.713	1.176	1.002	1.212	1.172	1.313	0.151	0.04	0.34
Manganese	0.030	0.036	0.040	0.036	0.037	0.030	0.004	0.76	0.74
Zinc	0.544	0.544	0.626	0.589	0.580	0.528	0.046	0.88	0.94
Heart									
Copper	0.060	0.066	0.078	0.068	0.071	0.062	0.006	0.86	0.58
Iron	0.898	1.120	1.207	1.179	1.142	1.151	0.123	0.42	0.51
Manganese	0.009	0.010	0.013	0.013	0.008	0.011	0.002	0.95	0.35
Zinc	0.486	0.489	0.601	0.530	0.516	0.460	0.046	0.54	0.80

[1]Tissue samples were collected from piglets in the third- and fourth-parity litters.
[2]Linear response to copper level ($P = 0.004$).
[3]Tendency of linear response to copper level ($P = 0.08$).

3.5 Discussion

3.5.1 Sow and litter performance

Pigs are Cu tolerant among domestic animal species (Hill and Spears, 2001) and high concentrations of dietary Cu (100 to 250 mg/kg) are commonly used in nursery and growing-finishing diets. But feeding nursery or growing pigs with higher dietary Cu levels (400 to 650 mg/kg) for more than 4 wk resulted in reduced gain and feed efficiency, reduced hemoglobin and hematocrit, reduced liver Fe levels, and even mortality (Bunch et al., 1965; DeGoey et al., 1971; Edmonds and Baker, 1986; Kornegay et al., 1989). However, Cu toxicosis has not been reported in pigs when dietary Cu level was less than 250 mg/kg. Sows have much longer lifespan than growing pigs. Thus pharmacological levels of Cu in sow diets might lead to a greater amount of Cu accumulation. In the present experiment, sows fed high Cu diets (120 and 220 mg/kg) for over 2 yr produced a numerically greater number of litters than sows fed 20 mg/kg Cu (38 and 37 vs. 31 litters; Table 3.4). This demonstrates that feeding high Cu in the long term did not have any negative effect on fertility of sows. This is in agreement with the results of Cromwell et al. (1993), who reported that there was no difference in the number of litters produced by sows fed diets containing 0 or 250 mg/kg of supplemental Cu (above the basal NRC level in the diets) as $CuSO_4$ for 6 parities.

In the present experiment, gestation weight gain was not affected by dietary Cu source or level for second- to fourth-parity sows (Table 3.5). Cromwell et al. (1993) reported a greater average 108-d weight of high Cu (250 mg/kg of supplemental Cu) fed sows than low Cu (0 mg/kg of supplemental Cu) fed sows ($P < 0.001$) throughout 6 parities,. Based on a review of the literature, it was concluded that 200 to 250 mg/kg of supplemental Cu

increased ADG and G:F of growing-finishing pigs (Cromwell, 2001). The improvement of gestation weight gain of the aforementioned study was speculated to be associated with the growth promoting effects of high Cu because gilts were gaining BW during the first parity. In the present experiment, the initial breeding weight was greater than that of the mentioned study (177.8 vs. 151.9 kg). Because the growth promoting effects of high Cu attenuated as pig BW increased (Cromwell, 2001), it might be possible to speculate that the growth rate was less responsive to high Cu for heavier than lighter gilts. Therefore, high Cu fed females in the present experiment did not show significantly greater gestation weight gain when compared to those fed with lower Cu diets.

With regard to lactation weight loss, no significant effect was detected on dietary Cu source or level in the present experiment. It is in agreement with the results reported by Thacker (1991), who demonstrated that sows top dressed with 0 or 250 mg/kg Cu from d 106 of gestation until the end of a 28-d lactation had similar lactation weight loss. However, the effects of high Cu on lactation weight loss was not consistent among previously published results. Cromwell et al. (1993) reported a greater lactation weight loss for sows fed diets with added 250 mg/kg Cu than those fed with 0 mg/kg Cu added diets.

Lactation daily feed intake tended to be greater for sows fed with TBCC diets than those fed with $CuSO_4$ diets ($P = 0.13$). Pharmacological levels of TBCC and $CuSO_4$ has been reported to increase feed intake of nursery and growing-finishing pigs by many researchers (Cromwell et al., 1998; Shelton et al., 2011; Coble et al., 2013b). Moreover, a feed intake preference study showed that finishing pigs (86.7 kg) preferred diets containing TBCC compared to $CuSO_4$, when given a choice between diets containing 150 mg/kg Cu from either source (Coble et al., 2013a). Because Cu is more concentrated in TBCC than $CuSO_4$,

it requires fewer moles of TBCC than $CuSO_4$ to provide the same mass of Cu. Therefore, TBCC introduces a lower amount of negative electrolyte (Cl^-) into diet than $CuSO_4$ (SO_4^{2-}) when supplementing the identical level of Cu, and consequently might result in less decline of dietary electrolyte balance (dEB). DeRouchey et al. (2003) concluded that sows fed with negative dEB diets (-200 and -100 mEq/kg) had much lower feed intake than those fed with positive dEB diets (0, 100, and 200 mEq/kg) during the first 7 d of lactation (2.4 vs. 5.3 kg/d). Furthermore, feed intake of positive dEB diet fed sows increased linearly ($P < 0.03$) as dEB values increased. The different response patterns of lactation daily feed intake to increasing dietary Cu levels between TBCC and $CuSO_4$ diets may be associated with the fact that TBCC had less impact on dEB than $CuSO_4$.

Increasing dietary Cu levels did not affect litter size of total born, live born, and weaning piglets ($P > 0.66$; Table 3.6) in the present experiment. Lillie and Frobish (1978) fed gilts with increasing supplemental Cu as $CuSO_4$ (0, 15, 30, and 60 mg/kg) throughout four reproductive cycles, and demonstrated that increasing dietary Cu levels did not affect litter size of total born, born alive, and weaning piglets. However, Cromwell et al. (1993) reported a significant improvement of litter size of total born for sows fed 250 mg/kg supplemented diets compared to sows fed diets without Cu supplementation ($P < 0.10$), but the litter size of live piglets at birth and weaning were not affected by dietary Cu level. Results of the present experiment were in agreement with previous studies. The number of stillborn piglets of sows in the present experiment was greater for sows fed TBCC diets than those fed $CuSO_4$ diets ($P = 0.03$). However, Cu source did not affect litter size of live born, and weaning piglets ($P > 0.33$), which indicated that the productivity of sows was not influenced by Cu source.

In the present experiment, increasing dietary Cu levels linearly increased piglet weight of total born (P = 0.12) and live born (P = 0.06). Meanwhile, liver Cu concentrations of sows also exhibited a linear increase with increasing dietary Cu levels (P < 0.0001; Table 3.19). A human study demonstrated that Cu concentrations in the placenta from 20 to 40 yr old mothers with 37 to 41 wk of gestation were positively correlated with neonate weight (Özdemir et al., 2009). These results might indicate an association between fetal development and maternal Cu status. Fetal development has been demonstrated to be manipulated by IGF-1 because it is involved in the regulation of nutrient transfer from mother to fetuses, as well as nutrient uptake and metabolism in fetuses (Gluckman and Pinal, 2003; Bowman et al., 2010). It has been reported that high dietary levels of Cu as $CuSO_4$ (100 to 300 mg/kg) increased serum IGF-1 level of growing pigs (Wang et al., 2016). Therefore, it is speculated that the improved piglet weight at birth might be associated with increased Cu status of sows, which might mediate fetal development through elevating circulating IGF-1 levels.

Significant interactions between dietary Cu source and level were observed on adjusted piglet weight at weaning (P = 0.06) and adjusted piglet weight gain (P = 0.02), with increasing linear response within TBCC treatments while no response or decreasing linear response within $CuSO_4$ treatments, to the increasing dietary Cu levels. Tribasic copper chloride has been demonstrated to be a less prooxidative form of Cu than $CuSO_4$ in the intestinal lumen of animals and in feed. It has been reported that the duodenal MDA levels were numerically lower in nursery pigs fed 225 mg/kg of Cu as TBCC than as $CuSO_4$ (Fry et al., 2012; Huang et al., 2015). Moreover, Luo et al. (2005) reported that the vitamin E content was greater in liver and plasma of broiler that were fed diets supplemented with

high levels of Cu (150, 300, and 450 mg/kg) as TBCC than those fed diets containing the same level of Cu as $CuSO_4$. In addition to the greater oxidative activity in the body, $CuSO_4$ has also been reported to exert greater prooxidant activity in diets. Lu et al. (2010) reported that poultry diets supplemented with 200 mg/kg Cu as $CuSO_4$ had significantly lower dietary vitamin E content ($P < 0.05$) after being stored at room temperature (18 ± 5°C) for 10 d, when compared to diets with the same level of added Cu as TBCC. Furthermore, the authors analyzed vitamin E contents in diets after being stored at room temperature for 41 d, and the results showed that there was 22% of vitamin E left in $CuSO_4$ diet compared to dietary vitamin E content before storage, whereas it was 74% for the TBCC diet and 71% for control diet (without Cu supplementation). In addition, Miles et al. (1998) reported that peroxide values and anisidine values of lipid extracted from stored poultry diets (supplemented with 25, 100, and 300 mg/kg Cu) were greater in $CuSO_4$ added diets compared to TBCC added diets over a 20-d storage time ($P = 0.0001$). Therefore, it is speculated that the greater oxidative activity of $CuSO_4$ might cause an increased oxidative status of sows, or damage of other nutrients in the sow diets; which would eventually affect lactation performance of sows and compromise litter performance. In the present experiment, milk from sows fed TBCC diets had greater levels of nutrients than that from sows fed $CuSO_4$ diets, which might support the speculation.

3.5.2 Apparent total tract digestibility

Apparent total tract digestibility of DM, nitrogen, and GE were similar between late gestation and lactating sows (Table 3.7 and 3.8); however, the ATTD of EE during lactation was 15% lower than that during late gestation ($P = 0.01$). Veum et al. (1995) reported that the ATTD of EE during lactation (d 14 to 21 of lactation) was significantly higher than that

during late gestation (d 90 to 97 of gestation). In this aforementioned study, the inclusion rate of dehydrated alfalfa meal was high in gestation diets but declined in lactation diets (25.0 vs. 12.5%), which resulted in reduction of 3.7% of NDF and 3.0% of ADF in lactation diets as compared to in gestation diets. However, the major difference between gestation and lactation diets in the present experiment was that about 9% of corn in the lactation diets were substituted by SBM, which indicated about 0.18% greater crude fiber in lactation diets than in gestation diets, based on the values in (NRC, 2012). In addition, the ATTD of Ca and P of sows in lactation increased by 41 and 33%, respectively, when compared to those values of late gestation sows. NRC (2012) suggests that the daily requirement of Ca and P for lactating sows (37.7 to 48.1 g/d of Ca; 18.9 to 24.0 g/d of P) are much greater than those for gestation sows (16.4 to 19.9 g/d of Ca; 12.5 to 14.8 g/d of P). The increase in requirement reflects the greater demands of Ca and P for milk production, and the greater ATTD of Ca and P might indicate that lactating sows upregulate absorption in an attempt to satisfy the demands for more Ca and P.

Sows fed TBCC diets had greater ATTD of DM, nitrogen, GE, Ca, and P than those fed $CuSO_4$ diets during lactation. It might suggest that TBCC fed sows absorbed more nutrients and consequently provide more nutrients to their progenies through milk. In the present experiment, piglets from TBCC fed sows had greater weaning weight and weight gain during lactation than those from $CuSO_4$ fed sows, which agreed with the inference.

During late gestation, the ATTD of Cu linearly increased ($P < 0.05$), whereas the ATTD of Fe tended to decrease linearly ($P < 0.10$) as dietary Cu level increased. Sharp (2004) stated that Cu and Fe did interact at the absorption sites, possibly through competition for transport into enterocytes via DMT1. Hedges and Kornegay (1973) demonstrated that Fe

content in liver and kidney was significantly lower in nursery pigs fed a diet containing 257 mg/kg Cu compared to those fed diets containing 7 and 25 mg/kg Cu for a period of 9 wk. In addition, a weaning-to-finishing study demonstrated that liver Fe concentration of pigs fed 120 and 240 mg/kg of Cu was 50 to 60% lower than those fed diets containing no more than 60 mg/kg of Cu (Bradley et al., 1983).

During lactation, the ATTD of Fe was not affected by dietary Cu source or level, but their values were negative for all treatments. This indicates that the amount of endogenous loss of Fe is greater than the amount of Fe absorbed through diets. In addition, the results of hemoglobin and hematocrit of sows in the present experiment showed about 10% reduction from late gestation to lactation (Table 3.10), which was in accordance with negative the ATTD of Fe during lactation.

In the present experiment, the ATTD of trace minerals ranged from -35 to 38% for late gestation and -52 to 39% for lactation. The wide range has resulted in a greater variation for the ATTD of trace minerals than that of most other nutrients (Table 3.7 and 3.8). The index method that was used in the present experiment is based on the assumptions that the index compound is completely indigestible, regularly and completely voided in the feces, and uniformly mixed with the feed and feces (Adeola, 2001), and with very high recovery rate. The analytical error of trace mineral analysis might be aggravated when these assumptions were not satisfied, and consequently yielded tremendous variation. The total collection method, which is used in many other studies to determine trace mineral digestibility in growing pigs (Korniewicz et al., 2007a; Deng et al., 2010; Lebel et al., 2014; Liu et al., 2014), might be less affected by such issues.

3.5.3 Antioxidant status of sows and piglets

The activity of total and Cu/Zn SOD in sow serum increased linearly with increasing dietary Cu levels regardless of Cu sources (Gestation, $P < 0.10$; Lactation, $P < 0.05$). Copper is a critical constituent of Cu/Zn SOD; it is reversibly oxidized and reduced by successive encounters with O_2^- to yield O_2 and H_2O_2 during SOD catalysis (Tainer et al., 1983). It has been reported that 50 and 250 mg/kg of supplemental Cu as $CuSO_4$ increased serum and erythrocyte SOD activity of growing pigs, respectively, when compared with pigs fed diets without supplemental Cu (Feng et al., 2007; Gonzales-Eguia et al., 2009). Moreover, increasing dietary Cu levels were also reported to increase hepatic SOD activity of beef cattle (40 vs. 0 mg/kg supplemental Cu) and serum Cu/Zn SOD activity of lipopolysaccharide challenged broiler chickens (50 vs. 0 mg/kg supplemental Cu) (Song et al., 2011; Correa et al., 2014).

It has been demonstrated that lactating sows had increased systemic oxidative stress as a greater lymphocyte DNA damage when compared to sows at d 30 of gestation (Berchieri-Ronchi et al., 2010). In the present experiment, serum MDA levels were on average 40% greater in lactating sows than in gestation sows. Moreover, the activity of serum antioxidant enzymes (total and Cu/Zn SOD, Cp) were greater in TBCC treatments than $CuSO_4$ treatments during lactation ($P < 0.10$). Meanwhile, serum MDA levels in lactating sows were not affected by dietary Cu source or levels in the present experiment. This indicates that compared with $CuSO_4$ fed sows, TBCC fed sows either produced greater amount/activity of antioxidant enzymes or had a lower oxidative status that requires less antioxidant enzymes to keep body oxidative homeostasis. It has been reported that TBCC fed animals had lower duodenal MDA levels, and higher hepatic and serum vitamin E

levels compared to $CuSO_4$ fed animals when pharmacological levels of Cu was used in the diets (Luo et al., 2005; Fry et al., 2012; Huang et al., 2015).

With regard to piglets at birth, total SOD activity (linear, $P = 0.07$) and Cu/Zn SOD activity (quadratic, $P = 0.09$) tended to increase as sow dietary Cu level increased. Meanwhile, the liver Cu concentration of piglets at birth showed a numerical increase as Cu levels in sow diets increased (Table 3.21). However, the regression analysis between SOD activity and liver Cu concentration at birth failed to detect significant correlation (data not shown).

Ceruloplasmin is one of the Cu-dependent proteins synthesized in the liver and acts as a primary Cu binding glycoprotein in blood plasma, accounting for 40 to 70% of total plasma Cu (Linder, 2016). In addition, it exerts SOD activity and scavenges free radicals in animals (Sang, 1995). In the present experiment, the activity of Cp was extremely low in the serum of neonatal piglets, but it increased by over 20-fold at weaning. Moreover, weanling piglets from TBCC fed sows had greater Cp activity ($P = 0.01$) than those from $CuSO_4$ fed sows. The greater Cp activity might indicate more circulating Cu and higher Cu status of weanling piglets, but Cu concentrations in liver, kidney, and heart of the weanling piglets did not show a difference between the Cu sources in the sow diets (Table 3.23).

The activity of total and Cu/Zn SOD in milk were found to decrease from 20 to 120 mg/kg Cu diets and then increased from 120 to 220 mg/kg Cu diets (quadratic, $P < 0.05$). Moreover, the estimated total and Cu/Zn SOD output in milk showed similar response trend (quadratic, $P < 0.10$; Table 3.15). Newborns are particularly prone to oxidative damage, and concentration and activity of antioxidants in milk determine the degree of protection from peroxidation (Kasapovic et al., 2005; Wilinska et al., 2015). The greater

SOD activity and output in milk from sows fed high Cu diets might indicate a better protection to their progenies. However, litter and piglet weight gain during lactation did not show any difference among dietary Cu levels.

3.5.4 Colostrum and milk composition

Milk from sows fed with TBCC diets contained greater levels of fat, protein, GE, total solids ($P < 0.10$), but a lower level of lactose ($P = 0.10$) than those fed with $CuSO_4$ diets. The greater nutrient levels in milk might explain the greater adjusted piglet weight at weaning, as well as the greater adjusted litter and piglet weight gain of litters from TBCC fed sows. In addition, the greater nutrient concentrations might indicate a more developed mammary gland; and consequently, more active nutrient synthesis in the mammary cells of TBCC fed sows. Mammary gland development occurs throughout many stages of growth and reproduction in swine, and various hormones are involved in the control of mammary development (Farmer, 2015). Growth hormone, which is secreted by the pituitary gland, regulates the development of mammary gland mainly through IGF-1 (Ruan and Kleinberg, 1999; Gallego et al., 2001). Zhou et al. (1994b) demonstrated that dietary Cu level of 215 mg/kg had increased GH mRNA concentrations ($P < 0.05$) of nursery pigs regardless of ad libitum or restricted feeding. Yang et al. (2011) reported that growing pigs fed with 125 mg/kg Cu as $CuSO_4$ for 45 d significantly increased growth hormone-releasing hormone and decreased somatostatin mRNA expression in the hypothalamus. Moreover, 100 mg/kg of Cu as $CuSO_4$ has been reported to increase serum IGF-1 levels of growing pigs (Wang et al., 2016). Therefore, it is speculated that the greater nutrient levels in milk produced by TBCC fed sows might be associated with the Cu-induced changes of hormone secretion.

Copper concentration and predicted Cu output in milk increased as dietary Cu level increased (linear, $P < 0.05$); meanwhile, Zn concentration (linear, $P < 0.05$) and predicted Zn output in milk decreased with increasing dietary Cu levels (quadratic, $P < 0.10$). Moreover, the liver Zn concentration and content of weanling pigs showed a tendency to decrease as Cu level increased in sow diets (linear, $P < 0.10$; Table 3.23 and 3.24). These results indicate that the Zn deposition in piglets has been compromised by declined Zn concentrations in milk. In the present experiment, Zn concentrations in liver, heart, kidney, and ovary of sows were not affected by dietary Cu source or level (Table 3.19). This suggests that the overall Zn status of sows were similar across treatments. Therefore, one may speculate that the decreased Zn concentration in milk is because the greater Cu status of sows depresses Zn uptake or transport in the mammary gland. However, there is no published data that support such speculation.

3.5.5 Tissue trace mineral levels

In the present experiment, the relative weight of liver ($P = 0.04$) and kidney ($P = 0.08$) was greater in sows that completed 3 or 4 parities than in baseline gilts. With the assumption that the slaughtered baseline gilts are representative for all gilts at the University of Kentucky Swine Research Center, the results imply that reproduction requires a more active nutrient catabolism and anabolism in the liver, as well as more metabolic waste excretion through the kidney. Consequently, resulted in a faster relative growth rate of these organs compared to BW.

Greater liver Cu concentration has been observed in sows than baseline gilts since more than two-thirds of observed sows received elevated levels of Cu in diets (120 and 220 vs. 20 mg/kg Cu diets). However, a separate single degree of freedom contrast revealed that

the liver Cu concentrations between 20 mg/kg Cu fed sows and baseline gilts did not differ from each other ($P > 0.35$). It is in agreement with previously published data, which demonstrated that liver Cu concentration did not differ between gilts or primiparous sows and multiparous sows that completed 2 to 7 parities (Peters et al., 2010; Crenshaw et al., 2013). In the present experiment, liver Mn and Zn concentrations of sows that completed 3 or 4 parities were greater than that of baseline gilts ($P < 0.05$). Peters et al. (2010) reported similar results, which showed that liver Mn (quadratic, $P = 0.01$) and Zn (cubic, $P = 0.01$) concentrations increased from parity 1 to 4, and then decreased from parity 4 to 6. Liver Fe concentrations of the present experiment did not differ between sows and baseline gilts; whereas Peters et al. (2010) showed that liver Fe concentrations decreased from parity 1 to 4, and then increased from parity 4 to 6 (quadratic, $P = 0.01$). However, the previous studies did not provide consistent results; Crenshaw et al. (2013) reported no statistical difference of liver Fe or Zn concentrations among sows from parity 0 to 7.

In the present experiment, sows fed the TBCC diets had greater concentration and content of liver Cu compared to sows fed the $CuSO_4$ diets ($P < 0.05$). The liver is critical for maintaining whole-body Cu homeostasis because more than 95% of the Cu excretion is via bile under normal physiological conditions (Roberts and Sarkar, 2008). Because the ATTD of dietary Cu was not affected by dietary Cu source, it is speculated that the greater concentration and content of liver Cu in $CuSO_4$ fed sows might be due to less Cu excretion. Prohaska (2008) stated that intracellular chaperone protein Atp7b is responsible for efflux of Cu to bile in the hepatocyte. It has been reported that weanling pigs fed a diet with 225 mg/kg supplemental Cu as TBCC for 12 d had numerically greater Atp7b mRNA expression in liver and greater Cu concentration in bile when compared to pigs fed a $CuSO_4$

diet (Huang et al., 2015). Copper concentration in sow bile could be used to validate such speculation; but unfortunately, bile samples were not collected in the present experiment.

Moreover, liver Fe concentrations of sows fed TBCC diets were greater than those from sows fed $CuSO_4$ diets in the present experiment ($P < 0.05$). High dietary Cu levels have been demonstrated to depress Fe deposition in liver and kidney of pigs (Hedges and Kornegay, 1973; Bradley et al., 1983), and the antagonism is speculated to be through competition for transport into enterocytes via DMT1 (Sharp, 2004). Divalent metal transporter 1 is the main transporter for divalent cations uptake in enterocytes; moreover, DMT1 is also expressed in hepatocytes to facilitate Fe uptake (Takami and Sakaida, 2011; Scheers, 2013). The greater Fe concentration in the liver for TBCC fed sows than $CuSO_4$ fed sows is suspected to be due to less antagonism between Fe and Cu in hepatocytes.

Trace mineral concentrations and contents of neonatal piglets did not differ across treatments. This indicates that transfer of trace minerals from the uterus to fetuses was not affected by dietary Cu source and level. However, liver Cu concentrations and contents of weanling piglets linearly increased as sow dietary Cu level increased ($P < 0.05$). This is associated with Cu concentrations in milk, which exerted a significant linear increase with increasing sow dietary Cu levels. However, because the suckling piglets could access sow feed and feces, the greater Cu accumulation in the liver might also be partially attributed to intake of sow feed and/or coprophagy.

3.5.6 Source of manganese and zinc in diets

In the present experiment, trace mineral premixes for TBCC diets contained Zn as zinc hydroxychloride and Mn as manganese hydroxychloride; whereas those for $CuSO_4$ diets contained Zn as zinc sulfate monohydrate and Mn as manganese sulfate monohydrate.

There is limited information reported on the relative bioavailability of zinc and manganese hydroxychloride to their sulfate counterparts for domestic animals. A feed preference study on post-weaning beef calves showed that a diet that contained manganese hydroxychloride was preferred to manganese sulfate when given a choice between diets containing 3,000 mg/kg Mn from either source (Caramalac et al., 2017). However, EFSA (2016) concluded that manganese hydroxychloride did not affect Mn deposition in tissues compared to manganese sulfate, and the use of manganese hydroxychloride in animal feed is safe for consumers. It has been reported that 100 mg/kg of Zn as zinc hydroxychloride did not affect growth performance of growing-finishing pigs (Carpenter et al., 2016a). In addition, a beef cattle study demonstrated that zinc hydroxychloride did not affect feedlot performance, carcass merit, or liver Zn status of yearling steers compared to zinc sulfate or zinc amino acid complex (Wagner et al., 2016). In the present experiment, total content of Mn and Zn in various organs of sows and piglets did not differ between TBCC and $CuSO_4$ treatments. Therefore, it is assumed that the differences between Zn and Mn source had no impact on the results of the current experiment.

3.6 Implication

In the current experiment, sows fed with TBCC diets had improved nutrient digestibility during lactation, lower Cu but greater Fe and Mn concentrations in liver, higher nutrient levels in milk, higher antioxidant enzyme activity during lactation, and greater litter performance, when compared to sows fed with $CuSO_4$ diets. Additionally, increasing dietary Cu levels linearly increased digestibility of DM, GE, and Cu during lactation, the antioxidant enzyme activity of sows, nutrient levels in milk, Cu deposition in sow liver, total born and live born piglet weight, and liver Cu concentrations and contents in weanling

piglets. Results of the present experiment indicate that TBCC is a superior Cu source compared to $CuSO_4$ regarding reproductive performance; the feeding of a high Cu diet did not have any apparent negative effects on sows and progenies, and higher Cu levels in sow diets resulted in the greater birth weight of piglets. Further research to investigate the effects of feeding high Cu diets to lactating sows only, or the effects of adding organic sources of Cu with reduced levels might be needed.

CHAPTER 4. Effects of Dietary Copper Levels on Growth Performance and Response to Lipopolysaccharide Challenge in Nursery Pigs from Sows Fed Either High or Low Copper Diets

4.1 Abstract

The objective of the present experiment was to determine the effects of dietary copper (Cu) levels on growth performance and response to lipopolysaccharide challenge of nursery pigs from sows fed high or low Cu diets. In a 28-d growth trial, a total of 32 crossbred weanling pigs (19.9 ± 2.2 d; initial BW of 5.96 ± 0.65 kg) were used in a 2 × 2 factorial arrangement with a split-plot design, with the main plot being sow dietary Cu level (20 or 120 mg/kg) and the subplot being nursery dietary Cu level (20 or 220 mg/kg). Pigs were blocked by BW and sex, and then randomly allotted to pens; pens were randomly assigned to 1 of 2 nursery diets. There were 2 pigs per pen and 4 pens (2 male pens and 2 female pens) per treatment. Upon completing the growth trial on d 28, 1 pig from each pen was moved to a new pen whereupon all experimental pigs were singly housed. After deprivation of feed for 12 h, the pigs were weighed and given an intraperitoneal injection of either phosphate-buffered saline or lipopolysaccharides (LPS, 50 μg/kg of BW) solution at an equal volume of 5 mL. The weight of pigs and feeders, rectal temperature, respiratory rate, and blood samples were monitored or collected at multiple time points post-injection. Pigs from sows fed with 120 mg/kg Cu diets had greater ADG from d 0 to 7 and d 0 to 14 ($P < 0.05$), and tended to have greater ADG for the overall period ($P < 0.08$), when compared to pigs from sows that were fed 20 mg/kg Cu diets. Body weight change and feed intake of LPS challenged pigs were not affected by treatment effect from 0 to 168 h post-injection. The challenged pigs from sows fed 120 mg/kg Cu had a greater overall

rectal temperature than those from sows fed 20 mg/kg Cu (P = 0.01). Also, the challenged pigs fed with 220 mg/kg Cu diets had greater serum tumor necrosis factor-alpha over time as compared to those fed with 20 mg/kg Cu diets (P = 0.03). The results of the present experiment demonstrated that high Cu levels in sow and nursery diets promoted growth performance of nursery pigs and affected their responses to the immunological challenge.

Key Words: copper, sows, nursery pigs, lipopolysaccharide, immune response

4.2 Introduction

Pigs reared in commercial facilities have to cope with immunological stressors from infectious agents or vaccination (Martínez-Miró et al., 2016). Perpetual exposure to immune stimulation results in the production of pro-inflammatory cytokines that repartition nutrients away from anabolic growth to support highly prioritized immunological pathways, and thus suppress the growth of pigs (Klasing and Johnstone, 1991; Spurlock, 1997). In-feed administration of antibiotics is a common practice to improve growth performance of pigs, and a proposed mechanism for the growth-promoting ability of antibiotics has been through preventing immunological stress (Roura et al., 1992; Cromwell, 2002). Unfortunately, antibiotic application to feed for growth promotion has been banned in the European Union and the US (EUROPA, 2003; FDA, 2013) due to the potential risks of antimicrobial resistance in human pathogenic bacteria (Kim et al., 2005). In the past 2 decades, an intensive amount of research has been focused on the development of alternatives to antibiotics, and copper (Cu) is one of the most competitive candidates.

Cromwell (2001) concluded that high levels of Cu (200 to 250 mg/kg) could improve growth performance of growing pigs. Additionally, high Cu diets have been reported to enhance reproductive performance of sows (Thacker, 1991; Cromwell et al., 1993). Moreover, Klasing and Barnes (1988) reported that growing chicks challenged with lipopolysaccharides (LPS) resulted in increased serum Cu levels; and a recent study showed that mice administrated with LPS had increased serum Cu levels but decreased liver Cu levels as compared to saline administrated mice (Han et al., 2013). These results might suggest that immunologically stressed animals mobilize body Cu reserve. Therefore, the objective of the present experiment was to assess the effects of dietary Cu levels on

growth performance and responses to LPS challenge of nursery pigs from sows fed high or low Cu diets.

4.3 Experimental procedures

This experiment was carried out in environmentally controlled rooms at the University of Kentucky Swine Research Center. The experimental protocols were approved by the Institutional Animal Care and Use Committee of the University of Kentucky.

4.3.1 Animals, housing, management, and experimental design

In a 28-d growth trial, a total of 32 crossbred weanling pigs [16 barrows and 16 gilts; Yorkshire × Duroc; (Yorkshire × Duroc) × Yorkshire; (Yorkshire × Landrace × Duroc) × Yorkshire] that were weaned at 19.9 ± 2.2 d (initial BW of 5.96 ± 0.65 kg) were used in a 2 × 2 factorial arrangement with a split-plot design, with the main plot being sow dietary Cu level (20 or 120 mg/kg) and the subplot being nursery dietary Cu level (20 or 220 mg/kg). The experimental treatments were: T1) 20 mg/kg sow dietary Cu and 20 mg/kg nursery dietary Cu, T2) 20 mg/kg sow dietary Cu and 220 mg/kg nursery dietary Cu, T3) 120 mg/kg sow dietary Cu and 20 mg/kg nursery dietary Cu, and T4) 120 mg/kg sow dietary Cu and 220 mg/kg nursery dietary Cu. The experimental pigs were selected from 2 sows (parity 2 and 4) that were fed diets that contained 120 mg/kg Cu as tribasic copper chloride (TBCC) or copper sulfate ($CuSO_4$), and 6 sows (parity 3 to 7) that were fed diets that contained 20 mg/kg Cu as $CuSO_4$. All sows were fed diets containing amino acids, vitamins, and minerals that met or exceeded NRC (2012) requirement estimates for gestation and lactating sows (Table 3.1 and 3.2).

At weaning, pigs within each main-plot factor were blocked by BW and sex, and then randomly allotted to pens. Pens were then randomly assigned to 1 of 2 nursery diets

containing either 20 or 220 mg/kg of Cu as $CuSO_4$. There were 2 pigs per pen and 4 pens (2 male pens and 2 female pens) per treatment. The animal allotment was accomplished using the Experimental Animal Allotment Program (Kim and Lindemann, 2007). All pigs were housed in 1.22 × 1.22 m^2 raised-deck nursery pens with plastic coated expanded metal flooring in an environmental-controlled room. Each pen was equipped with a nipple waterer and a single-sided, 3–hole plastic and metal feeder. Pigs were allowed to have ad libitum access to feed and water throughout the experiment.

Upon completing the growth trial on d 28, 1 pig from each pen was moved to a new pen in another environmental-controlled nursery room. Thus all experimental pigs were singly housed after d 28. All pigs were deprived of feed from 1800 h on d 28 until 0600 h on d 29 when the LPS challenge period started. The 2 pigs from each original pen were weighed and given an intraperitoneal injection of either phosphate-buffered saline (PBS) or lipopolysaccharide (LPS) solution at an equal volume of 5 mL that provided 50 μg LPS per kg of BW. The LPS (Escherichia coli serotype 0111:B4 phenol extract; Sigma L-2630, Sigma-Aldrich, Inc., St. Louis, MO) was dissolved in autoclaved PBS solution (P-4417, Sigma-Aldrich, Inc., St. Louis, MO) to make 0.5 mg/mL LPS solution. A volume of 5 mL was prepared for each pig by combining PBS with the appropriate volume of LPS solution. The heavier or lighter pig of the original pen alternatively received LPS injection within treatment. Pigs were suspended by the rear legs, and the solution was injected into the peritoneal cavity, three nipples down from the caudal end and approximately 3 cm lateral to the nipple on the left side of the pig. After injection, pigs were allowed to have ad libitum access to the same diets as in the growth trial. There were equal numbers of pigs that received either LPS or PBS in each nursery room.

4.3.2 Experimental diets

One basal diet was formulated for each of two 14-d phases with nutrient levels that met or exceeded the NRC (2012) requirement estimates (Table 4.1). The treatment diets were made by supplementing Cu (0 or 200 mg/kg) in the form of $CuSO_4$ to basal diets at the expense of corn. Experimental diets were in mash form.

4.3.3 Data and sample collection

4.3.3.1 Growth trial

Pigs and feeders were weighed on d 0, 7, 14, 21, and 28 to calculate ADG, ADFI, and G:F. About 2 mL of blood was collected in 13 × 75 mm vacutainer tubes coated with K_2EDTA (Becton, Dickinson and Company, Franklin Lakes, NJ) on d 0, 14, and 28.

4.3.3.2 Lipopolysaccharide challenge

Pigs and feeders were weighed about 5 min before injection, and at 2, 4, 6, 8, 10, 12, 24, 48, 72, 96, 120, 144, and 168 h post-injection. Rectal temperature was measured by a digital veterinary thermometer, and blood sample was collected via jugular venipuncture about 5 min before injection, and at 2, 4, 6, 8, 10, and 12 h post-injection. Respiratory rate was visually measured by counting flank movements for 1 min of the resting pigs about 30 min before injection, and at 1, 3, 5, 7, 9, and 11 h post- injection. Blood samples were immediately placed on ice and then transported to the laboratory. Serum samples were obtained by centrifugation at 1700 × g for 20 minutes at 4°C; and then aliquoted into 1.5 mL Eppendorf Safe-Lock Tubes (Eppendorf North America, Hauppauge, NY), flash frozen in liquid nitrogen, and stored at −80°C until analysis.

Table 4.1. Composition of basal diets (as-fed basis)

	Basal diets	
Item	Phase 1	Phase 2
Ingredient, %		
Corn	50.47	61.66
Soybean meal, 48% CP	30.00	33.00
Fish meal	1.50	0.00
Spray-dried animal plasma	2.00	0.00
Dried whey	10.00	0.00
Grease, choice white	3.10	2.10
L-Lysine·HCl	0.25	0.32
DL-Methionine	0.18	0.16
L-Threonine	0.09	0.12
Dicalcium Phosphate	1.11	1.08
Limestone	0.63	0.89
Salt	0.40	0.40
Trace mineral premix[1]	0.15	0.15
Vitamin premix[2]	0.10	0.10
Santoquin[3]	0.02	0.02
Total	100.00	100.00
Calculated composition		
Metabolizable energy, kcal/kg	3,401	3,351
Lysine, %[4]	1.35	1.23
Methionine + Cysteine, %[4]	0.81	0.73
Threonine, %[4]	0.85	0.77
Calcium, %	0.80	0.70
Phosphorous, %[5]	0.40	0.33

[1]Supplied the following per kilogram of diet: 50 mg as manganese sulfate monohydrate; 100 mg of Fe as ferrous sulfate monohydrate; 125 mg of Zn as zinc sulfate monohydrate; 20 mg of Cu as copper sulfate; 0.35 mg of I as calcium iodate; and 0.30 mg of Se as sodium selenite.

[2]Supplied the following per kilogram of diet: 11,000 IU of vitamin A; 1,100 IU of vitamin D3; 77 IU of vitamin E; 2.2 mg of vitamin K; 0.03 mg of vitamin B12; 8.25 mg of riboflavin; 27.50 mg of pantothenic acid; 30.25 mg of niacin; 4.95 mg of folic acid; 4.95 mg of vitamin B6; 1.65 mg of thiamin; and 0.36 mg of biotin.

[3]Santoquin (Monsanto, St. Louis, MO) supplied 130 mg/kg ethoxyquin in the diet.

[4]Calculated composition of amino acids is on the standardized ileal digestible basis.

[5]Calculated composition of phosphorus is on the standardized total tract digestible basis.

4.3.4 Laboratory analysis

The whole blood samples were collected in tubes containing K_2EDTA as an anticoagulant and were measured for hematocrit (Htc) and hemoglobin (Hb) within 3 h after collection. Hematocrit was determined using standard hematocrit tubes and centrifugation, and Hb was measured using a commercial assay kit and Hb standard (Pointe Scientific, Inc., Canton, MI) according to the manufacturer's instructions.

The frozen serum samples were allowed to thaw at room temperature for chemical analysis. The cortisol concentrations in the serum samples were determined using coated tube radioimmunoassay (MP Biomedicals, LLC., Orangeburg, NY). Standards or serum samples and radiolabeled cortisol (^{125}I) were sequentially added to antibody-coated tubes. After incubation in a 37°C water bath for 45 minutes, all moisture in the coated tube was thoroughly removed, and the radioactivity was measured using a Packard Cobra Auto-gamma Counter (Packard Instrument Co., Meriden, CT). Interleukin-6 (IL-6) and tumor necrosis factor-alpha (TNF-α) in serum samples were analyzed by commercial enzyme-linked immunosorbent assay kits specific for the quantitative measurements of porcine IL-6 and TNF-α (RayBiotech, Inc., Norcross, GA) according to the manufacturer's instructions. All the laboratory assays were conducted in duplicate.

4.3.5 Statistical analysis

All data were analyzed using the PROC MIXED procedure in SAS (Statistical Analysis System, Cary, NC, USA) for a split-plot design. The main plot was the sow dietary Cu level, and the subplot was the Cu level in the nursery diets. The pen served as the experimental unit and results are reported as least squares means. The statistical model included fixed effects of sow dietary Cu level, nursery dietary Cu level, and their

interaction, as well as random effects of block and block by sow dietary Cu level interaction. The block × sow dietary copper level interaction was used as the error term to test for the effects of sow dietary copper level, and the residual error was used as the error term to test for the effects of nursery dietary copper level. The model is shown as follows:

$Y_{ijk} = \mu + B_i + S_j + N_k + (B \times S)_{ij} + (S \times N)_{ik} + e_{ijk}$

Y = response variables (growth performance; BW and feed intake during the challenge period; vital signs; serum cortisol and cytokines)

μ = constant common to all observations

B_i = block effect

S_j = sow dietary copper level effect

N_k = nursery dietary copper level effect

$(B \times S)_{ij}$ = block × sow dietary copper level interaction

$(S \times N)_{ik}$ = sow dietary copper level × nursery dietary copper level interaction

e_{ijk} = error term of the model

Least squares means separation was accomplished by using the PDIFF option of SAS. One pig from the treatment of 20 mg/kg sow Cu level and 20 mg/kg of nursery Cu level was removed from the experiment because of weight loss in the growth trial. The feed intake of that pen was corrected with the Feed Intake Correction Spreadsheet (Lindemann and Kim, 2007). With regard to data analysis for the LPS challenge study, statistical outliers for feed intake (n=1, treatment 3), IL-6 (n=2, treatment 2 and 4), and TNF-α (n=1, treatment 2) were identified using the Grubb's test outlier calculator (GraphPad Software, San Diego, CA, USA) and removed from statistical analysis. In addition, data for the LPS challenge study were also analyzed as repeated measures to determine the response trends

to injection over time (Littell et al., 1998). Statistical differences were considered significant at $P < 0.05$ and tendency at $P < 0.10$.

4.4 Results

4.4.1 Growth trial

The results for the growth performance are presented in Table 4.2. The greater Cu level in sow diets resulted in greater BW of pigs at d 7, 14, 21 ($P < 0.05$), and d 28 of the experiment ($P = 0.06$). However, the BW of pigs was not affected by the effect of nursery Cu level or interaction between sow and nursery dietary Cu level ($P > 0.16$). Pigs from T4 had the numerically greatest BW among all treatments on d 7, 14, 21, and 28 of the experiment, and were heavier ($P < 0.05$) than pigs from T1 on these same days.

Pigs from sows fed with 120 mg/kg Cu diets had greater ADG from d 0 to 7 and Phase 1 ($P < 0.05$), as well as tended to have greater ADG for the overall period ($P < 0.08$) when compared to pigs from sows fed 20 mg/kg Cu . Dietary Cu levels in the nursery diets did not affect ADG of pigs, but an interaction was observed for d 14 to 21 ADG ($P = 0.04$) between Cu levels in sow and nursery diets. Pigs fed the 220 mg/kg Cu diet tended to have improved ADG ($P = 0.06$) compared to those fed the 20 mg/kg Cu diet within the 20 mg/kg sow Cu treatments; whereas nursery dietary Cu levels did not affect ADG of those pigs from the 120 mg/kg Cu fed sows ($P = 0.46$). Although lacking significance, the same pattern was also observed on overall ADG ($P = 0.19$). Overall ADG showed a 12% improvement when pigs from sows fed 20 mg/kg Cu were provided with 220 mg/kg compared to 20 mg/kg Cu in the nursery diets (470 vs. 421 g/d); but pigs from sows fed 120 mg/kg Cu had similar overall ADG between nursery Cu levels (492 vs. 491 g/d). Pigs

from T4 had the numerically greatest ADG among all treatments during Phase 1 and the overall period, and was greater ($P < 0.05$) than pigs from T1 in the same periods.

Pigs from sows fed 120 mg/kg Cu tended to consume more feed from d 0 to 7 than those from sows fed 20 mg/kg Cu ($P = 0.08$); however, there was no difference on ADFI across treatments thereafter. Pigs from sows fed diets that contained 120 mg/kg Cu had greater G:F than those from sows fed 20 mg/kg Cu in Phase 1 ($P = 0.02$) and for the overall period ($P = 0.07$). Pigs from T4 had the numerically greatest G:F among all treatments during Phase 1 and the overall period, and was greater ($P < 0.05$) than pigs from T1 in the same periods. Hemoglobin and hematocrit were not affected by sow or nursery dietary Cu levels throughout the experiment ($P > 0.25$, Table 4.3). Also, both hematologic indexes linearly increased ($P < 0.0001$) as days advanced in the experiment.

Table 4.2. Effects of sow and nursery dietary copper levels (mg/kg) on growth performance of nursery pigs

Sow copper level:	20	20	120	120			
Nursery copper level:	20	220	20	220		*P* values	
Item Treatment No.:	1	2	3	4	SEM	Sow	Nursery
No. of observations	4	4	4	4			
BW, kg							
d 0	5.96	5.92	6.01	6.01	0.32	0.48	0.54
d 7	6.67^{c}	6.91^{bc}	7.32^{ab}	7.52^{a}	0.44	0.03	0.23
d 14	9.62^{b}	10.20^{ab}	10.84^{a}	11.06^{a}	0.69	0.03	0.19
d 21	13.07^{b}	14.36^{ab}	15.11^{a}	15.11^{a}	0.85	0.04	0.16
d 28	17.69^{b}	19.08^{ab}	19.76^{a}	19.77^{a}	1.05	0.06	0.21
ADG, g							
d 0 to 7	111^{b}	141^{ab}	188^{ab}	216^{a}	27	0.05	0.26
d 7 to 14	421^{b}	470^{ab}	502^{ab}	505^{a}	41	0.08	0.25
d 14 to 21[1]	494^{b}	595^{a}	610^{a}	580^{ab}	29	0.13	0.20
d 21 to 28	664	675	666	665	38	0.90	0.86
Phase 1 (d 0 to 14)	265^{b}	306^{ab}	345^{a}	361^{a}	28	0.04	0.19
Phase 2 (d 14 to 28)	577	635	638	622	31	0.33	0.34
Overall (d 0 to 28)	421^{b}	470^{ab}	491^{a}	492^{a}	27	0.07	0.19
ADFI, g							
d 0 to 7	203	206	246	277	30	0.08	0.47
d 7 to 14	498	558	542	559	48	0.45	0.21
d 14 to 21	742	855	852	822	52	0.43	0.37

Continued

Table 4.2 continued

d 21 to 28	983	996	1,039	990	59	0.53	0.63
Phase 1 (d 0 to 14)	350	382	394	418	36	0.16	0.26
Phase 2 (d 14 to 28)	861	926	946	906	54	0.43	0.74
Overall (d 0 to 28)	605	654	670	662	15	0.25	0.47
G:F							
d 0 to 7	0.522[b]	0.655[ab]	0.775[ab]	0.788[a]	0.068	0.08	0.29
d 7 to 14	0.844	0.850	0.928	0.905	0.023	0.06	0.74
d 14 to 21	0.662	0.698	0.720	0.705	0.019	0.17	0.60
d 21 to 28	0.669	0.678	0.640	0.673	0.017	0.44	0.21
Phase 1 (d 0 to 14)	0.757[c]	0.800[bc]	0.880[ab]	0.860[a]	0.021	0.02	0.60
Phase 2 (d 14 to 28)	0.667	0.688	0.678	0.685	0.014	0.78	0.33
Overall (d 0 to 28)	0.692[b]	0.720[ab]	0.738[ab]	0.743[a]	0.013	0.07	0.23

[a–c]Least squares means within the same row without a common superscript differ ($P < 0.05$).

[1]Sow dietary copper level × nursery dietary copper level interaction ($P = 0.04$). Nursery dietary copper effect tended to be significant ($P = 0.06$) within the 20 mg/kg sow dietary Cu treatment whereas no effect within the 120 mg/kg Cu treatment ($P = 0.46$).

Table 4.3. Effects of sow and nursery dietary copper levels (mg/kg) on hematocrit and hemoglobin levels of nursery pigs

Sow copper level:	20	20	120	120			
Nursery copper level:	20	220	20	220		*P* values	
Item Treatment No.:	1	2	3	4	SEM	Sow	Nursery
No. of observations	4	4	4	4			
Hemoglobin, g/dL[1]							
d 0	10.23	9.72	11.05	10.34	0.67	0.36	0.40
d 14	11.56	10.99	11.76	11.66	0.36	0.35	0.25
d 28	13.04	12.99	13.81	13.46	0.52	0.40	0.61
Hematocrit, %[1]							
d 0	30.24	28.97	33.10	30.23	1.75	0.32	0.28
d 14	34.25	32.71	33.21	33.48	0.69	0.82	0.27
d 28	36.07	35.97	37.54	36.88	0.99	0.40	0.61

[1]Orthogonal polynomial contrasts indicated that hemoglobin and hematocrit increased (linear, $P < 0.0001$) as days advanced.

4.4.2 Lipopolysaccharide challenge

4.4.2.1 Effects of phosphate buffered saline or lipopolysaccharide injection

Figure 4.1(A) shows the effects of LPS and PBS injection on the BW of the experimental pigs. Pigs injected with LPS had lower BW during the first 12 h post-injection, but the PBS injected pigs had greater BW during the same period; all experimental animals were gaining weight thereafter until the final weighing at 168 h post-injection and demonstrated an interaction between injection and time ($P < 0.0001$). Furthermore, the BW of PBS injected pigs was greater than that of LPS injected pigs at each time point from 6 to 168 h post-injection ($P < 0.05$); and PBS injected pigs had an overall greater BW than LPS injected pigs during the entire challenge period (Injection, $P = 0.05$).

With regard to BW change, pigs injected with LPS lost BW, whereas PBS injected pigs gained BW during the first 6 h post-injection; the BW change of the PBS injected pigs was greater than that of LPS injected pigs during the post-injection periods of 0 to 2 h, 2 to 4 h, and 4 to 6 h [$P < 0.05$; Figure 4.1(B)]. Moreover, PBS injected pigs had greater overall BW gain compared to that of LPS injected pigs (Injection, $P < 0.0001$); and a interaction between injection and time was observed on overall BW change ($P = 0.003$). Figure 4.1(C) shows that pigs injected with PBS had greater cumulative BW change at each post-injection time point ($P < 0.05$) and for the overall periods (Injection, $P < 0.0001$) than LPS injected pigs. Analysis of repeated measures also demonstrated a interaction between injection and time ($P = 0.0004$).

Feed intake of PBS injected pigs was greater than that of LPS injected pigs at each post-injection period except for the 144 to 168 h period [$P < 0.05$; Figure 4.2(A)]. Also, pigs injected with PBS had greater feed intake over time when compared to that of the LPS

injected pigs (Injection, $P = 0.006$). The cumulative feed intake [Figure 4.2(C)] of the PBS injected pigs was greater than that of the LPS injected pigs at each post-injection period ($P < 0.05$) and overall periods (Injection, $P = 0.009$).

The LPS injected pigs had greater rectal temperatures than those of the PBS injected pigs from 2 to 12 h post-injection ($P < 0.05$), and pigs injected with LPS had greater overall rectal temperature than the PBS injected pigs (Injection, $P = 0.008$; Figure 4.3). Moreover, an interaction between injection and time was observed on rectal temperature ($P < 0.0001$). In contrast, the overall response trend of respiratory rate [Figure 4.3(B)] was not affected by injection ($P = 0.16$) or injection × time interaction ($P = 0.18$).

The LPS injected pigs had peaks of serum cortisol, IL-6, and TNF-α at 2 or 4 h post-injection, and then gradually declined to pre-injection levels; while the PBS injected pigs had stable serum levels of circulating cortisol and cytokines throughout the experiment (Injection, $P < 0.0001$; Injection × Time, $P < 0.0001$; Figure 4.4). Pigs injected with LPS had greater IL-6 at 2, 4, and 6 h post-injection, as well as greater cortisol and TNF-α from 2 to 12 h post-injection ($P < 0.05$).

In summary, the LPS injected pigs had smaller BW gain and feed intake, higher rectal temperature, and greater circulating cortisol and cytokine levels during the challenge period as compared to those of the PBS injected pigs, demonstrating that the LPS injected pigs had been immunologically stimulated in the present experiment. Detailed data of pig response to LPS or PBS injection are presented in Appendix 6.

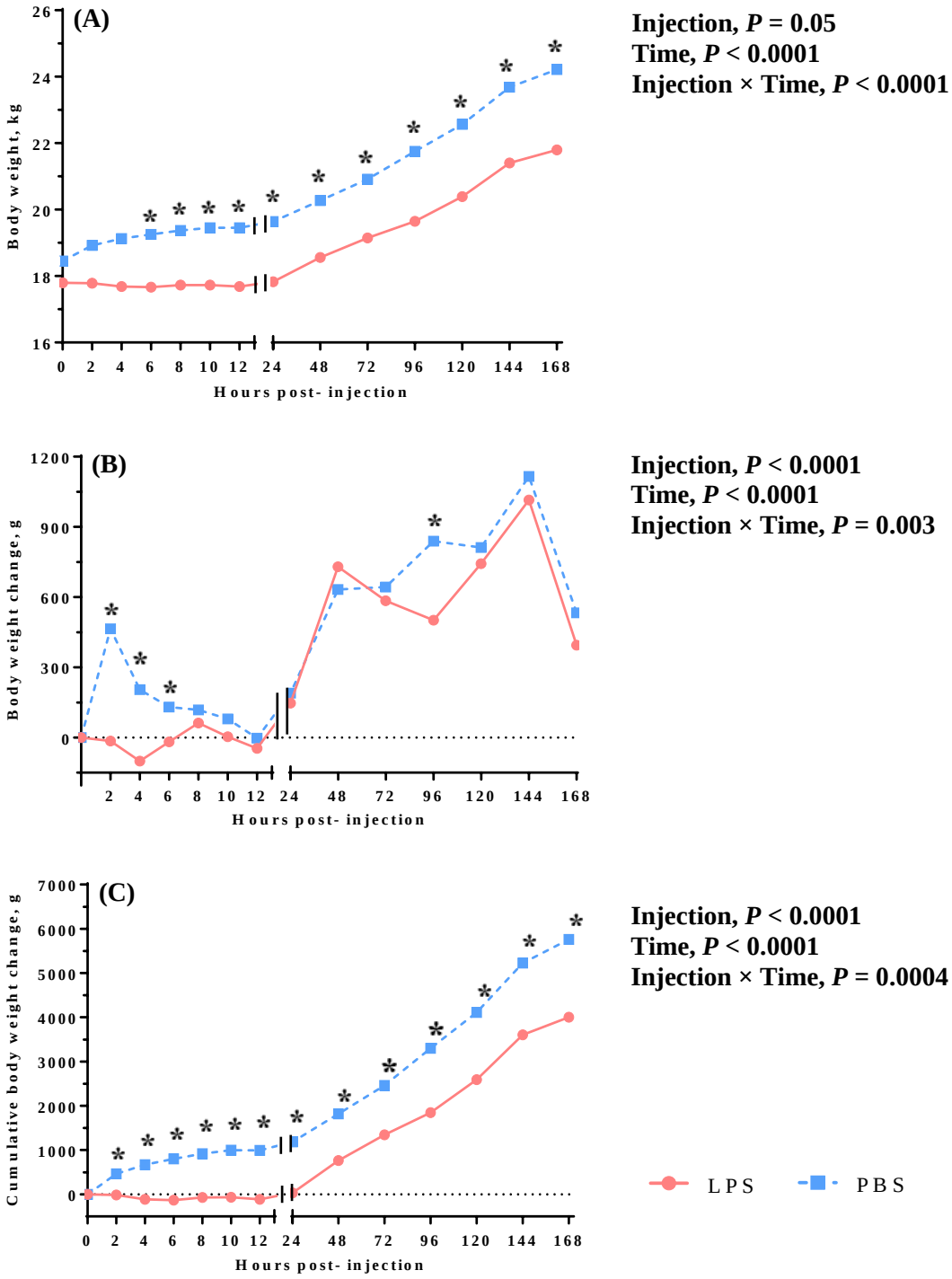

Figure 4.1. Effects of phosphate buffered saline (PBS, n = 15) or lipopolysaccharide (LPS, n = 16) injections on body weight (A), body weight change (B), and cumulative body weight change (C) of pigs. At 0 h, 5 mL of LPS or PBS solution was intraperitoneally injected. The volume of LPS solution was determined to deliver 50 μg/kg BW. *Least squares means of LPS injected pigs is different from that of PBS injected pigs at a given period ($P < 0.05$).

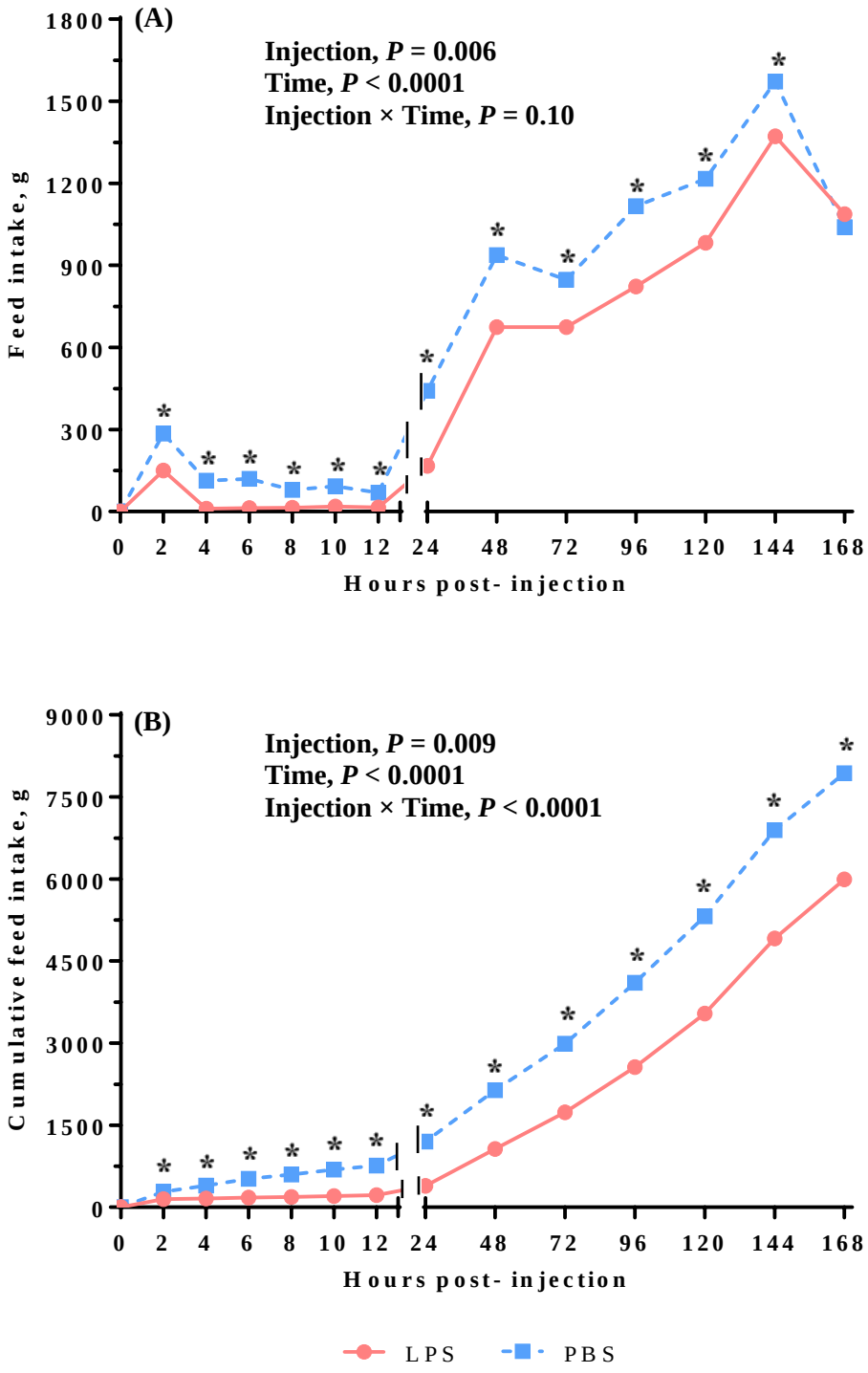

Figure 4.2. Effects of phosphate buffered saline (PBS, n = 15) or lipopolysaccharide (LPS, n = 16) injections on feed intake (A) and cumulative feed intake (B) of pigs. At 0 h, 5 mL of LPS or PBS solution was intraperitoneally injected. The volume of LPS solution was determined to deliver 50 μg/kg BW. *Least squares means of LPS injected pigs is different from that of PBS injected pigs at a given period ($P < 0.05$).

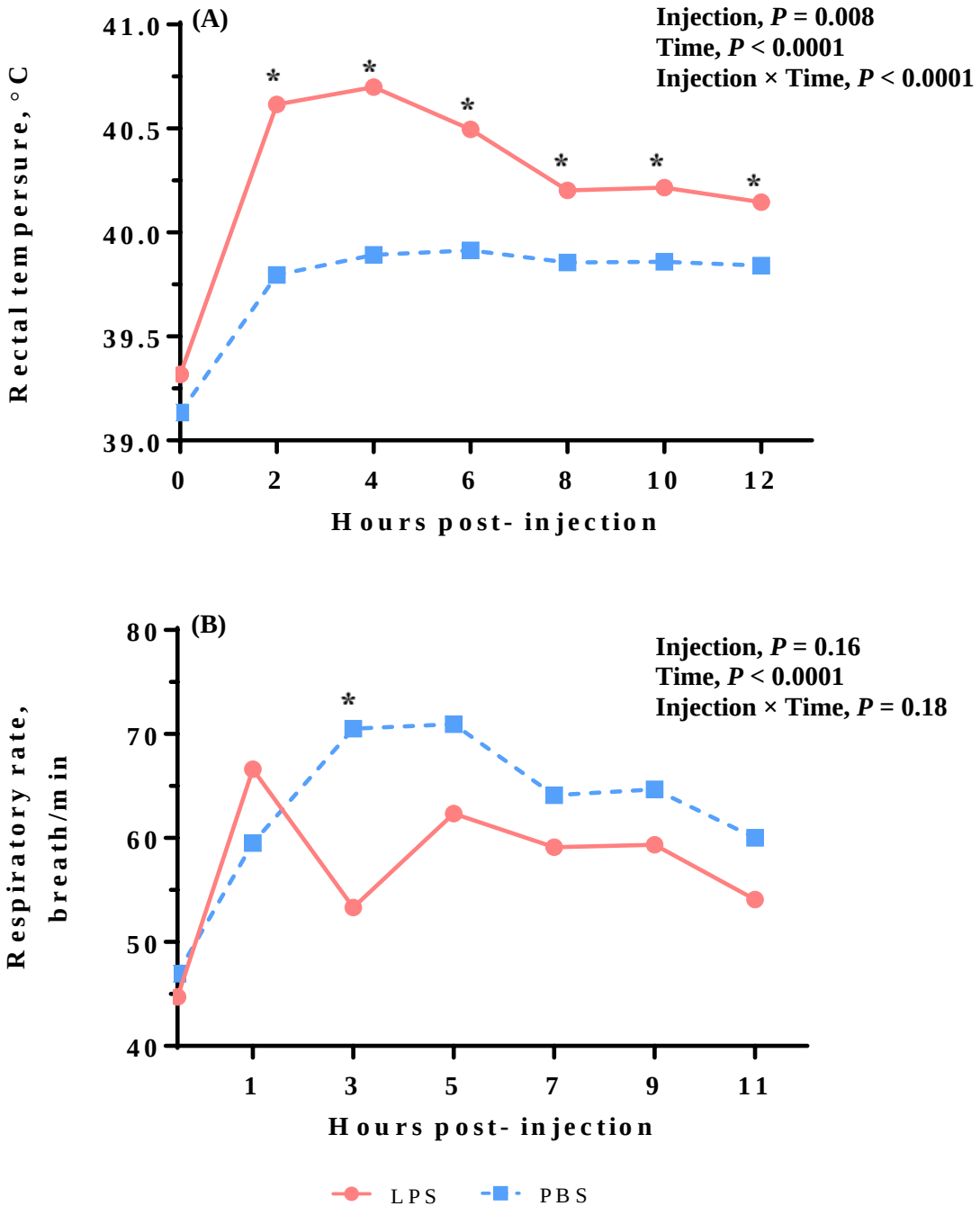

Figure 4.3. Effects of phosphate buffered saline (PBS, n = 15) or lipopolysaccharide (LPS, n = 16) injections on rectal temperature (A) and respiratory rate (B) of pigs. At 0 h, 5 mL of LPS or PBS solution was intraperitoneally injected. The volume of LPS solution was determined to deliver 50 μg/kg BW. *Least squares means of LPS injected pigs is different from that of PBS injected pigs at a given period ($P < 0.05$).

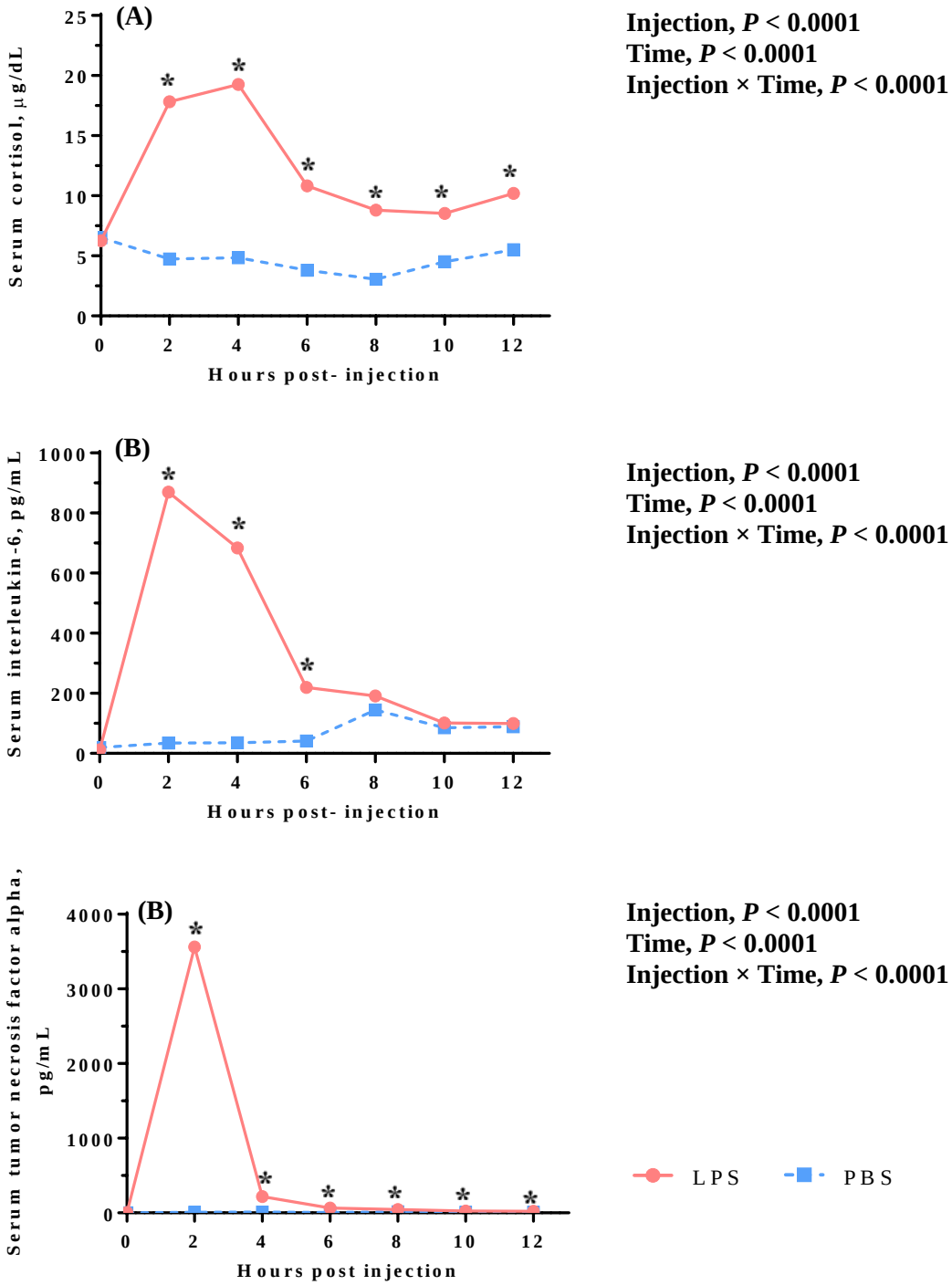

Figure 4.4. Effects of phosphate buffered saline (PBS, n = 15) or lipopolysaccharide (LPS, n = 16) injections on concentrations of serum cortisol (A), interleukin-6 (B), and tumor necrosis factor-alpha (C) of pigs. At 0 h, 5 mL of LPS or PBS solution was intraperitoneally injected. The volume of LPS solution was determined to deliver 50 µg/kg BW. *Least squares means of LPS injected pigs is different from that of PBS injected pigs at a given period ($P < 0.05$).

4.4.2.2 Effects of sow and nursery copper level

Body weight and feed intake

Body weight of pigs from sows fed 120 mg/kg Cu tended to be greater than those from 20 mg/kg Cu fed sows at each time point from 0 to 12 h post-injection ($P < 0.10$; Table 4.4). In addition, analysis of repeated measures showed that pigs from sows fed 120 mg/kg Cu tended to have greater BW than those from sows fed 20 mg/kg Cu from 0 to 12 h post-injection (acute phase). Although BW of pigs from 24 to 168 h post-injection was not statistically affected by sow dietary Cu level ($P > 0.11$), pigs from sows fed 120 mg/kg Cu had at least 5.8% greater BW than those from sows fed 20 mg/kg Cu during these specific time points. The nursery dietary Cu levels did not affect pig BW throughout the challenge period.

Table 4.5 and 4.6 shows BW change and cumulative BW change of LPS challenged pigs from 0 to 168 h post-injection. All pigs challenged with LPS had BW loss from 0 to 12 h post-injection, and the majority of the BW loss took place during 2 to 4 h post-injection. After 12 h post-injection, all pigs started to gain weight. Body weight change and cumulative BW change of pigs were not influenced by sow or nursery dietary Cu levels ($P > 0.08$). Furthermore, the repeated measure analysis indicated that there was no difference on response trends over time across dietary treatments ($P > 0.49$).

Feed intake of the challenged pigs during the first 2 h post-injection was greater than each of the subsequent 2-h periods until 12 h post-injection (Table 4.7). Repeated measure analysis from 0 to 12 h post-injection showed that pigs from sows fed 120 mg/kg Cu tended to have greater feed intake than those from sows fed 20 mg/kg Cu over time ($P = 0.10$). The cumulative feed intake of 0 to 12 h was numerically greater for pigs from sows fed 20

mg/kg Cu than those from sows fed 120 mg/kg Cu, but lacking significant difference (P = 0.27, Table 4.8). After 12 h post-injection, feed intake of challenged pigs gradually increased and reached to around 1,000 g per 24 h during 72 to 96 h post-injection, which was close to the feed intake level during the last week of the growth trial (Table 4.2). In the present experiment, feed intake and cumulative feed intake of each post-injection period or overall periods were not influenced by nursery dietary Cu levels (P > 0.23) throughout the experiment.

Table 4.4. Effects of sow and nursery dietary copper levels (mg/kg) on body weight of lipopolysaccharide challenged pigs

	Sow copper level:	20	20	120	120			
	Nursery copper level:	20	220	20	220		*P* values	
Item	Treatment No.:	1	2	3	4	SEM	Sow	Nursery
No. of observations		4	4	4	4			
Body weight, kg[1, 2]								
0 h		16.64[b]	17.25[ab]	18.94[a]	18.36[ab]	0.81	0.07	0.99
2 h		16.73	17.19	18.90	18.31	0.79	0.08	0.93
4 h		16.61[b]	17.17[ab]	18.81[a]	18.14[ab]	0.79	0.08	0.94
6 h		16.60[b]	17.18[ab]	18.84[a]	18.05[ab]	0.76	0.08	0.87
8 h		16.63[b]	17.29[ab]	18.96[a]	18.03[ab]	0.77	0.08	0.84
10 h		16.62[b]	17.30[ab]	18.92[a]	18.09[ab]	0.76	0.09	0.92
12 h		16.62[b]	17.23[ab]	18.88[a]	18.01[ab]	0.76	0.10	0.84
24 h		16.94	17.28	18.95	18.15	0.82	0.11	0.74
48 h		17.51	18.20	19.67	18.86	0.92	0.12	0.93
72 h		18.07	18.85	20.09	19.57	0.87	0.13	0.82
96 h		18.55	19.49	20.48	20.07	0.85	0.15	0.67
120 h		19.31	20.27	21.23	20.75	0.85	0.15	0.72
144 h		20.30	21.30	22.42	21.60	0.90	0.17	0.89
168 h		20.70	21.54	22.81	22.15	0.99	0.14	0.89

[a, b]Least squares means within the same row without a common superscript differ ($P < 0.05$).

[1]Analysis of repeated measures for 0 to 168 h shows significant time effects ($P < 0.0001$), but no significant effect for sow or nursery dietary copper level ($P > 0.12$) or their interaction ($P > 0.44$).

[2]Analysis of repeated measures for 0 to 12 h only shows significant time effects ($P < 0.0001$), tendency of sow copper effect ($P = 0.07$), but no significant effect for nursery dietary copper level ($P = 0.92$) or their interaction ($P > 0.31$).

Table 4.5. Effects of sow and nursery dietary copper levels (mg/kg) on body weight change of lipopolysaccharide challenged pigs

Sow copper level:	20	20	120	120			
Nursery copper level:	20	220	20	220		*P* values	
Item Treatment No.:	1	2	3	4	SEM	Sow	Nursery
No. of observations	4	4	4	4			
Body weight change, g[2, 3]							
0 to 2 h	88	-53	-45	-50	84	0.52	0.39
2 to 4 h	-123	-23	-93	-165	47	0.32	0.78
4 to 6 h	-15	10	30	-98	49	0.51	0.27
6 to 8 h	30	115	120	-13	60	0.77	0.70
8 to 10 h	-10	5	-40	55	99	0.92	0.56
10 to 12 h	3	-75	-35	-78	31	0.59	0.08
12 to 24 h	319	58	69	143	226	0.76	0.66
24 to 48 h	575	916	717	711	247	0.91	0.51
48 to 72 h	558	645	429	706	176	0.87	0.28
72 to 96 h	477	642	383	505	137	0.35	0.22
96 to 120 h	762	780	755	676	109	0.65	0.79
120 to 144 h	991	1,034	1,186	848	130	0.98	0.27
144 to 168 h[1]	398[ab]	243[b]	387[ab]	550[a]	135	0.13	0.95

[a, b]Least squares means within the same row without a common superscript differ ($P < 0.05$).

[1]Sow dietary copper level × nursery dietary copper level ($P = 0.03$).

[2]Analysis of repeated measures for 0 to 168 h shows significant time effects ($P < 0.0001$), but no significant effect for sow or nursery dietary copper level ($P > 0.55$) or their interaction ($P > 0.56$).

[3]Analysis of repeated measures for 0 to 12 h only shows significant time effects ($P < 0.0001$), but no significant effect for sow or nursery dietary copper level ($P > 0.36$) or their interaction ($P > 0.22$).

Table 4.6. Effects of sow and nursery dietary copper levels (mg/kg) on cumulative body weight change of lipopolysaccharide challenged pigs

	Sow copper level:	20	20	120	120			
	Nursery copper level:	20	220	20	220		*P* values	
Item	Treatment No.:	1	2	3	4	SEM	Sow	Nursery
No. of observations		4	4	4	4			
Cumulative weight change, g[1, 2]								
0 to 2 h		88	-52	-45	-50	84	0.51	0.40
0 to 4 h		-34	-75	-137	-217	114	0.36	0.62
0 to 6 h		-46	-67	-109	-313	142	0.36	0.46
0 to 8 h		-17	48	11	-328	180	0.41	0.48
0 to 10 h		-27	57	-29	-272	228	0.52	0.74
0 to 12 h		-26	-18	-65	-350	256	0.52	0.61
0 to 24 h		293	40	5	-207	365	0.52	0.55
0 to 48 h		867	956	721	503	523	0.61	0.91
0 to 72 h		1,425	1,601	1,150	1,210	494	0.55	0.82
0 to 96 h		1,902	2,243	1,533	1,714	525	0.46	0.64
0 to 120 h		2,664	3,023	2,288	2,390	531	0.41	0.68
0 to 144 h		3,655	4,057	3,474	3,238	595	0.46	0.89
0 to 168 h		4,053	4,299	3,861	3,788	607	0.60	0.89

[1]Analysis of repeated measures for 0 to 168 h shows significant time effects ($P < 0.0001$), but no significant effect for sow or nursery dietary copper level ($P > 0.55$) or their interaction ($P > 0.78$).

[2]Analysis of repeated measures for 0 to 12 h only shows significant time effects ($P < 0.0001$), but no significant effect for sow or nursery dietary copper level ($P > 0.45$) or their interaction ($P > 0.32$).

Table 4.7. Effects of sow and nursery dietary copper levels (mg/kg) on feed intake of lipopolysaccharide challenged pigs

Sow copper level:	20	20	120	120			
Nursery copper level:	20	220	20	220		*P* values	
Item / Treatment No.:	1	2	3	4	SEM	Sow	Nursery
No. of observations	4	4	4	4			
Feed intake, g[1, 2]							
0 to 2 h	146	192	155	108	42	0.47	0.98
2 to 4 h	18	12	1	12	11	0.49	0.80
4 to 6 h	18	27	5	2	16	0.33	0.84
6 to 8 h	28	25	1	1	19	0.27	0.93
8 to 10 h	46	19	8	0	25	0.33	0.51
10 to 12 h	45	9	7	1	17	0.27	0.27
12 to 24 h	252	143	137	136	112	0.63	0.64
24 to 48 h	693	825	546	635	167	0.39	0.53
48 to 72 h	683	791	545	677	89	0.25	0.23
72 to 96 h	884	995	667	747	106	0.12	0.40
96 to 120 h	1,069	1,107	880	873	93	0.11	0.88
120 to 144 h	1,390	1,463	1,387	1,249	105	0.37	0.77
144 to 168 h	931	864	754	1,683	471	0.52	0.37
Cumulative feed intake, g[1, 3]							
0 to 2 h	146	192	155	108	42	0.47	0.98
0 to 4 h	164	204	156	120	47	0.40	0.97
0 to 6 h	183	231	161	122	57	0.33	0.93

Continued

Table 4.7 continued

0 to 8 h	211	256	162	124	71	0.29	0.96
0 to 10 h	257	276	170	124	90	0.28	0.88
0 to 12 h	303	285	177	125	105	0.27	0.75
0 to 24 h	554	427	314	261	187	0.36	0.65
0 to 48 h	1,247	1,253	861	896	325	0.34	0.95
0 to 72 h	1,930	2,044	1,406	1,573	378	0.28	0.72
0 to 96 h	2,814	3,040	2,073	2,320	444	0.20	0.61
0 to 120 h	3,883	4,146	2,952	3,193	514	0.16	0.64
0 to 144 h	5,273	5,609	4,339	4,442	598	0.18	0.73
0 to 168 h	6,204	6,473	4,901	6,125	904	0.43	0.45

[1]Analysis of repeated measures for 0 to 168 h shows significant time effects ($P < 0.0001$), but no significant effect for sow or nursery dietary copper level ($P > 0.46$) or their interaction ($P > 0.17$).

[2]Analysis of repeated measures for 0 to 12 h only shows significant time effects ($P < 0.0001$), tendency of sow copper effect ($P = 0.10$), but no significant effect for nursery dietary copper level ($P = 0.68$) or their interaction ($P > 0.25$).

[3]Analysis of repeated measures for 0 to 12 h only shows significant time effects ($P < 0.0001$), but no significant effect for sow or nursery dietary copper level ($P > 0.31$) or their interaction ($P > 0.29$).

Vital signs

Rectal temperature of LPS challenged pigs increased from 0 to 2 or 4 h post-injection and declined thereafter until 12 h post-injection. However, the rectal temperature at 12 h post-injection did not return to the pre-injection levels, with an average elevation of 0.83°C (Table 4.8). The LPS challenged pigs from sows fed 120 mg/kg Cu had higher rectal temperature at 0, 2, and 4 h post-injection ($P < 0.05$), and tended to have a greater rectal temperature increase from 0 to 2 h post-injection ($P = 0.09$), when compared to those from sows fed 20 mg/kg Cu. Pigs fed 20 mg/kg Cu diet tended to have the higher rectal temperature at 2 h post-injection ($P = 0.07$) and greater rectal temperature decline ($P = 0.08$) during 4 to 6 h post-injection when compared to pigs that were fed 220 mg/kg diet. An interaction between sow and nursery dietary Cu level was detected for rectal temperature at 0 h post-injection ($P = 0.07$), and for rectal temperature change from 6 to 8 h ($P = 0.06$) and 8 to 10 h ($P = 0.03$) post-injection. In addition, pigs from T3 had the numerically highest rectal temperature at 0, 2, and 4 h post-injection. Analysis of repeated measures showed that pigs from sows fed 120 mg/kg Cu had a greater overall rectal temperature than those from sows fed 20 mg/kg Cu during the challenge period ($P = 0.01$; Figure 4.5a). The nursery dietary Cu levels or the interaction between sow and nursery dietary Cu levels did not affect the response curve of rectal temperature over time ($P > 0.61$).

Pigs from sows fed 120 mg/kg Cu tended to have a higher respiratory rate than those from sows fed 20 mg/kg Cu before LPS injection ($P = 0.06$; Table 4.9). Moreover, pigs fed 220 mg/kg Cu diet tended to have a higher respiratory rate than pigs fed 20 mg/kg diet at 9 h post-injection ($P = 0.06$). Also, respiratory rate change of the LPS challenged pigs was

not significantly affected by sow or nursery dietary Cu level, or their interaction. Analysis of repeated measures showed that pigs from sows fed 120 mg/kg Cu tended to have a higher overall respiratory rate than those from sows fed 20 mg/kg Cu during the challenge period ($P = 0.08$; Figure 4.5b). The nursery dietary Cu levels or the interaction between sow and nursery dietary Cu levels did not affect the response curve of respiratory rate over time ($P > 0.30$).

Table 4.8. Effects of sow and nursery dietary copper levels (mg/kg) on rectal temperature of lipopolysaccharide challenged pigs

Sow copper level:	20	20	120	120			
Nursery copper level:	20	220	20	220		*P* values	
Item Treatment No.:	1	2	3	4	SEM	Sow	Nursery
No. of observations	4	4	4	4			
Rectal temperature, °C							
0 h[2]	39.04[b]	39.14[b]	39.81[a]	39.28[b]	0.14	0.05	0.18
2 h	40.36[bc]	40.13[c]	41.21[a]	40.76[ab]	0.16	0.02	0.07
4 h	40.45[bc]	40.38[c]	41.17[a]	40.81[ab]	0.16	0.02	0.13
6 h	40.33	40.32	40.60	40.74	0.19	0.14	0.71
8 h	40.20	39.96	40.09	40.57	0.21	0.31	0.56
10 h	40.09	40.03	40.22	40.53	0.17	0.17	0.48
12 h	40.03	39.95	40.32	40.29	0.21	0.22	0.80
Rectal temperature change, °C[3]							
0 to 2 h	1.32[ab]	0.99[b]	1.40[a]	1.49[a]	0.14	0.09	0.32
2 to 4 h	0.09	0.25	-0.04	0.04	0.12	0.19	0.26
4 to 6 h	-0.11[ab]	-0.05[a]	-0.57[b]	-0.07[a]	0.13	0.17	0.08
6 to 8 h[2]	-0.14	-0.36	-0.51	-0.17	0.13	0.52	0.65
8 to 10 h[1]	-0.11	0.07	0.14	-0.04	0.08	0.51	0.97
10 to 12 h	-0.05	-0.08	0.10	-0.24	0.10	0.98	0.13

[a-c]Least squares means within the same row without a common superscript differ ($P < 0.05$).

[1]Sow dietary copper level × nursery dietary copper level ($P = 0.03$).

[2]Tendency of sow dietary copper level × nursery dietary copper level ($P < 0.10$).

[3]Analysis of repeated measures shows significant time effects ($P < 0.0001$) and interaction between sow dietary copper level and time ($P = 0.04$), and a tendency of interaction between nursery dietary copper level and time ($P = 0.09$); but no significant effect was detected on sow or nursery dietary copper level ($P > 0.57$) or their interaction ($P = 0.25$).

Table 4.9. Effects of sow and nursery dietary copper levels (mg/kg) on respiratory rate of lipopolysaccharide challenged pigs

Sow copper level:	20	20	120	120			
Nursery copper level:	20	220	20	220		*P* values	
Item Treatment No.:	1	2	3	4	SEM	Sow	Nursery
No. of observations	4	4	4	4			
Respiratory rate, breath/min							
-0.5 h	39.38	40.13	49.25	50.13	3.48	0.06	0.82
1 h	52.88	70.63	66.88	76.13	13.67	0.53	0.36
3 h	45.00	53.88	51.25	63.13	8.26	0.42	0.26
5 h	58.75	51.75	70.38	68.50	9.36	0.27	0.60
7 h	59.88	52.88	58.75	65.00	8.74	0.64	0.96
9 h	53.75[ab]	56.38[ab]	49.88[b]	77.38[a]	8.62	0.32	0.06
11 h	56.75	40.88	49.38	69.38	13.09	0.46	0.86
Respiratory rate change, breath/min[1]							
-0.5 to 1 h	13.50	30.50	17.63	26.00	14.09	0.99	0.40
1 to 3 h	-7.88	-16.75	-15.63	-13.00	15.13	0.90	0.84
3 to 5 h	13.75	-2.13	19.13	5.38	7.48	0.45	0.09
5 to 7 h	1.13	1.13	-11.63	-3.50	9.48	0.39	0.63
7 to 9 h	-6.13	3.50	-8.88	12.38	11.65	0.80	0.15
9 to 11 h	3.00	-15.50	-0.50	-8.00	9.68	0.85	0.23

[a-c]Least squares means within the same row without a common superscript differ ($P < 0.05$).

[1]Analysis of repeated measures shows significant time effects ($P = 0.01$), but no significant effect was detected on sow or nursery dietary copper level ($P > 0.94$), their interaction ($P = 0.36$), or interaction between time and treatment factor ($P > 0.24$).

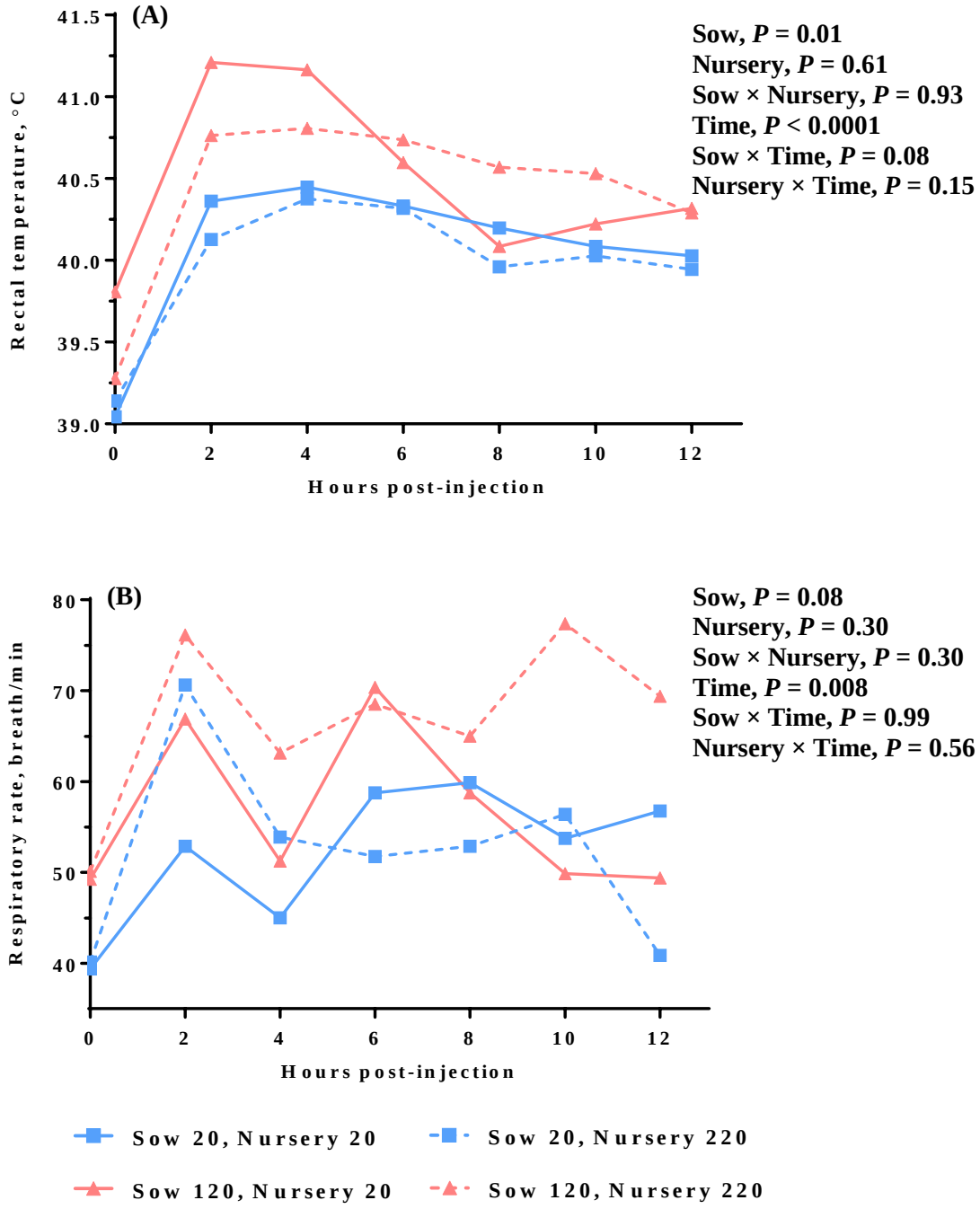

Figure 4.5. Effects of sow and nursery dietary copper levels on rectal temperature (A) and respiratory rate (B) of lipopolysaccharides challenged pigs. Each line represents 4 pigs (2 female and 2 male pigs. At 0 h, 5 mL of LPS or PBS solution was intraperitoneally injected. The volume of LPS solution was determined to deliver 50 µg/kg BW.

Serum concentrations of cortisol and cytokines

Serum cortisol levels were not affected by sow or nursery dietary Cu level at each sampling time point ($P > 0.15$; Table 4.10). However, the magnitude of serum cortisol increase from 0 to 2 h post-injection was greater for pigs from sows fed 120 mg/kg Cu than those from sows fed 20 mg/kg Cu ($P = 0.06$); and it was also greater for pigs fed with 220 mg/kg Cu diets compared to those that were fed 20 mg/kg Cu diets ($P = 0.04$). Furthermore, pigs from T4 had the numerically greatest cortisol increase from 0 to 2 h post-injection. Analysis of repeated measures showed no significant effect of sow or nursery dietary Cu level on serum cortisol levels or change of serum cortisol levels over time.

The results of serum IL-6 levels and IL-6 level change are presented in Table 4.11. Pigs from sows fed 120 mg/kg Cu tended to have a lower pre-injection level of IL-6 in serum compared to those from sows fed 20 mg/kg Cu ($P = 0.06$). Moreover, pigs from sows fed 120 mg/kg Cu tended to have greater serum IL-6 decline from 2 to 4 h post-injection compared to those from sows fed 20 mg/kg Cu ($P = 0.09$). Analysis of repeated measures showed no significant effect of sow or nursery dietary Cu level on serum IL-6 levels or change of serum IL-6 levels over time.

In the present experiment, pigs fed 220 mg/kg Cu diets had greater serum TNF-α levels at 2 h ($P = 0.06$) and 4 h ($P = 0.01$) post-injection compared to those fed 20 mg/kg Cu diets (Table 4.12). Furthermore, pigs fed 220 mg/kg Cu diets had greater magnitude of serum TNF-α increase from 0 to 2 h post-injection ($P = 0.06$); and greater magnitude of TNF-α decline from 2 to 4 h ($P = 0.07$) and 4 to 6 h ($P = 0.01$) post-injection when compared to pigs fed 20 mg/kg Cu diets. Serum TNF-α level or change of serum TNF-α levels were not affected by sow dietary Cu level at each sampling time point ($P > 0.18$). Analysis of

repeated measures showed that pigs fed with 220 mg/kg Cu diets had greater serum TNF-α levels over time when compared to those fed 20 mg/kg Cu diets ($P = 0.03$). In addition, interactions between sow or nursery dietary Cu level and time ($P < 0.05$) indicated different TNF-α response trends over time between low and high Cu levels in the sow and nursery diets.

Table 4.10. Effects of sow and nursery dietary copper levels (mg/kg) on serum concentration of cortisol of lipopolysaccharide challenged pigs

Sow copper level:	20	20	120	120			
Nursery copper level:	20	220	20	220		*P* values	
Item Treatment No.:	1	2	3	4	SEM	Sow	Nursery
No. of observations	4	4	4	4			
Cortisol, μg/dL[1]							
0 h	8.02	4.36	6.36	6.33	1.84	0.94	0.36
2 h	15.98	15.06	17.64	22.59	2.72	0.15	0.43
4 h	17.39	19.09	19.22	21.32	4.21	0.66	0.67
6 h	9.38	9.74	9.65	14.51	2.80	0.40	0.34
8 h	8.73	8.00	7.72	10.72	2.26	0.73	0.63
10 h	8.61	7.09	7.27	11.16	2.12	0.56	0.60
12 h	9.95	9.94	8.97	11.92	1.55	0.77	0.38
Cortisol change, μg/dL[1]							
0 to 2 h	7.97[b]	10.71[b]	11.28[ab]	16.25[a]	1.79	0.06	0.04
2 to 4 h	1.41	4.03	1.59	-1.27	2.12	0.31	0.96
4 to 6 h	-8.02	-9.35	-9.58	-6.81	2.51	0.87	0.77
6 to 8 h	-0.65	-1.74	-1.93	-3.79	1.66	0.36	0.37
8 to 10 h	-0.12	-0.92	-0.45	0.44	1.57	0.76	0.98
10 to 12 h	1.34	2.85	1.71	0.75	1.22	0.39	0.76

[a-c]Least squares means within the same row without a common superscript differ ($P < 0.05$).

[1]Analysis of repeated measures shows significant time effects ($P < 0.0001$), but no significant effect was detected on sow or nursery dietary copper level ($P > 0.35$), their interaction ($P > 0.31$), or interaction between time and treatment factor ($P > 0.21$).

Table 4.11. Effects of sow and nursery dietary copper levels (mg/kg) on serum concentrations of interleukin-6 of lipopolysaccharide challenged pigs

	Sow copper level:	20	20	120	120			
	Nursery copper level:	20	220	20	220		*P* values	
Item	Treatment No.:	1	2	3	4	SEM	Sow	Nursery
No. of observations		4	4	4	4			
Interleukin-6, pg/mL[1]								
0 h		12	26	7	7	4	0.06	0.15
2 h		825	998	700	1,072	296	0.94	0.38
4 h		835	953	399	644	364	0.41	0.62
6 h		276	256	155	306	171	0.89	0.39
8 h		231	188	127	291	86	0.99	0.31
10 h		158	123	63	127	59	0.59	0.66
12 h		147	94	51	109	52	0.48	0.97
Interleukin-6 change, pg/mL[1]								
0 to 2 h		814	972	693	1,065	297	0.97	0.39
2 to 4 h		9	-64	-301	-415	136	0.09	0.52
4 to 6 h		-559	-778	-245	-315	231	0.19	0.57
6 to 8 h		-44	-51	-28	-16	93	0.85	0.93
8 to 10 h		-73	-52	-63	-180	44	0.22	0.27
10 to 12 h		-11	15	-12	11	50	0.97	0.65

[1]Analysis of repeated measures shows significant time effects ($P < 0.0001$), but no significant effect was detected on sow or nursery dietary copper level ($P > 0.61$), their interaction ($P > 0.44$), or interaction between time and treatment factor ($P > 0.10$).

Table 4.12. Effects of sow and nursery dietary copper levels (mg/kg) on serum concentrations of tumor necrosis factor-alpha of lipopolysaccharide challenged pigs

Sow copper level:	20	20	120	120			
Nursery copper level:	20	220	20	220		*P* values	
Item Treatment No.:	1	2	3	4	SEM	Sow	Nursery
No. of observations	4	4	4	4			
Tumor necrosis factor-alpha, pg/mL							
0 h	10	3	8	2	3	0.64	0.12
2 h	2,680	7,267	1,574	3,651	1,396	0.19	0.06
4 h	157	339	145	281	75	0.74	0.01
6 h	46	66	49	101	28	0.58	0.21
8 h	33	55	44	54	15	0.80	0.19
10 h	26	15	22	34	12	0.68	0.96
12 h	23	14	21	29	12	0.66	0.94
Tumor necrosis factor-alpha change, pg/mL[1]							
0 to 2 h	2,670^{ab}	7,264^{a}	1,567^{b}	3,649^{ab}	1,397	0.19	0.06
2 to 4 h	-2,522^{ab}	-6,951^{b}	-1,429^{a}	-3,370^{ab}	1,353	0.18	0.07
4 to 6 h	-111	-272	-95	-180	54	0.50	0.01
6 to 8 h	-13	-9	-5	-47	24	0.60	0.40
8 to 10 h	-7	-40	-23	-20	14	0.91	0.23
10 to 12 h	-3	1	0	-5	4	0.78	0.99

$^{a-c}$Least squares means within the same row without a common superscript differ ($P < 0.05$).

[1]Analysis of repeated measures shows significant time effects ($P < 0.0001$), interaction between sow dietary copper level and time ($P = 0.01$), and interaction between nursery dietary copper level and time ($P = 0.0001$); but no significant effect was detected on sow or nursery dietary copper level ($P > 0.99$), or their interaction ($P = 0.99$).

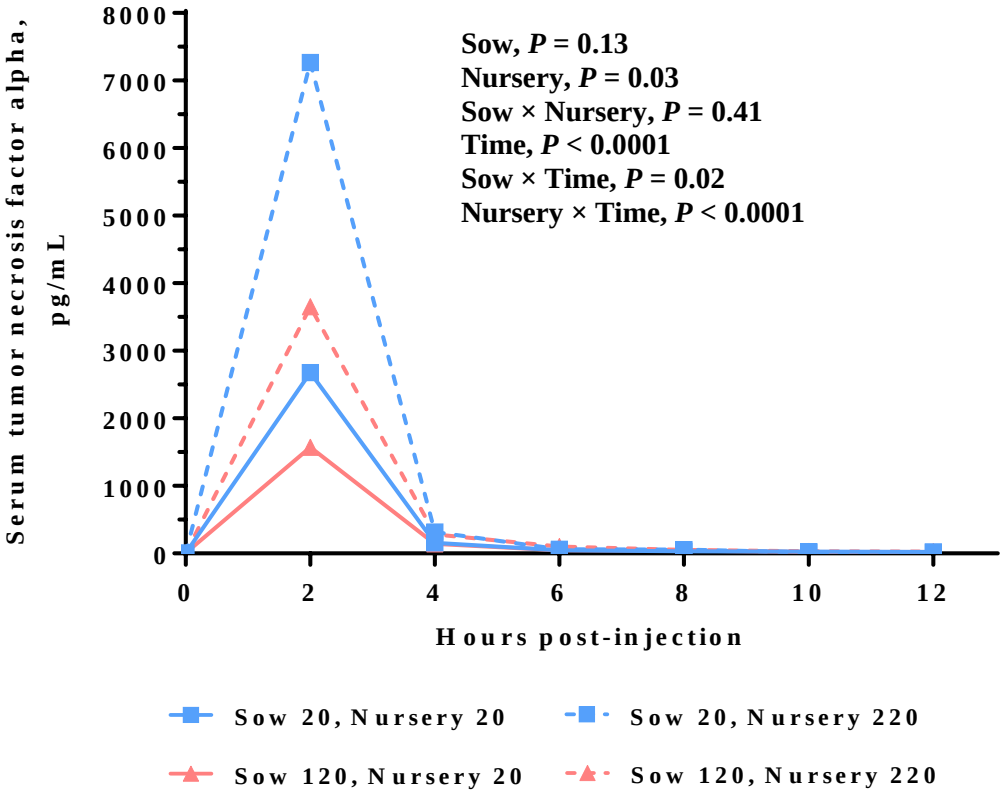

Figure 4.6. Effects of sow and nursery dietary copper levels on serum tumor necrosis factor-alpha of lipopolysaccharides challenged pigs. Each line represents 4 pigs (2 female and 2 male pigs). At 0 h, 5 mL of LPS or PBS solution was intraperitoneally injected. The volume of LPS solution was determined to deliver 50 μg/kg BW.

4.5 Discussion

4.5.1 Growth performance

In the present experiment, pigs from sow 1841 (120 mg/kg Cu as TBCC; Parity 4) and sow 1881 (120 mg/kg Cu as $CuSO_4$; Parity 2) were used for the high Cu fed sow groups. According to the results of Chapter 3, concentrations and contents of liver trace minerals (Cu, Fe, Mn, and Zn) of weanling pigs were not affected by maternal dietary Cu sources ($P > 0.29$) or sow parity ($P > 0.18$). Therefore, pigs from sows fed 120 mg/kg Cu were assumed homogeneous with regard to liver trace mineral status.

The growth promoting effects of pharmacological levels of Cu on pigs have been extensively evaluated during the past decades. Cromwell (2001) concluded that nursery and growing pigs provided with high Cu diets (200 to 250 mg/kg Cu as $CuSO_4$) had an average improvement of 11.9 and 6.9% in ADG, as well as 4.5 and 3.6% improvement in feed/gain ratio when compared to pigs fed diets containing 6 to 12 mg/kg of Cu. Moreover, high dietary Cu supplementation is already a common practice in commercial production. A survey of 18 US swine production systems representing 40% of the US sow herd revealed that an average level of 160 mg/kg of Cu is being supplemented in nursery diets (weaning to 23 kg of BW) (Flohr et al., 2016a). However, overall ADG and G:F of the present experiment did not show any difference between nursery Cu levels ($P > 0.19$). Shurson et al. (1990b) fed germ-free or conventionally reared weanling pigs with either low (16 mg/kg) or high (283 mg/kg) Cu diets for 3 wk, and reported that germ-free pigs tended to have greater ADG than conventionally reared pigs (average of 211 vs. 114 g/d). However, the high dietary Cu level tended to reduce ADG in germ-free pigs (223 vs. 195 g/d) but tended to improve ADG in conventionally reared pigs (102 vs. 126 g/d). In addition,

hematology results showed that conventionally reared pigs had greater leukocyte counts than that of germ-free pigs ($P = 0.01$); and the high Cu diet reduced leukocyte counts by 21 and 6% in conventional reared and germ-free pigs, respectively. These results indicate that the presence of environmental microorganisms compromised the growth rate of pigs, and pharmacological levels of Cu exerts antimicrobial-like growth promoting effects by alleviating the stress from those environmental microorganisms. The University of Kentucky Swine Research Center has routine management to thoroughly clean the nursery rooms with disinfectant agents and a high-pressure washer between experiments to maintain environmental hygiene. It is speculated that experimental pigs were exposed to a less stressful environment compared to those in commercial settings. Therefore, nursery Cu levels did not exhibit growth-promoting effects similar to many reported studies in the current experiment.

However, an interaction between maternal and nursery Cu levels on d 14 to 21 ADG ($P = 0.04$) demonstrated that the high nursery Cu level was effective at improving the growth rate of pigs from sows fed 20 mg/kg Cu, but not for those from sows fed 120 mg/kg Cu. Although lacking a significant interactive effect, Phase 1 and overall ADG showed similar response patterns across treatments as that of the d 14 to 21 ADG. Zhou et al. (1994a) parenterally administrated increasing dosages of Cu histidinate to weanling pigs parenterally and reported that increasing Cu dosages increased ADG and serum mitogenic activity (quadratic, $P < 0.05$), and elevated serum, liver, and brain Cu concentrations (linear, $P < 0.05$). This clearly demonstrates that a mode of action that does not involve antimicrobial activity exists. Furthermore, Zhou et al. (1994b) demonstrated that weanling pigs pair-fed a Cu-supplemented diet (215 mg/kg) to the level of pigs fed a control diet (no

supplemental Cu) had numerically greater ADG and G:F, greater growth hormone mRNA concentrations in pituitary ($P < 0.05$), and increased Cu concentrations in serum, liver, and brain ($P < 0.05$) compared to the control pigs. Moreover, the authors stated that the Cu-stimulated growth might be dependent on a direct action of Cu on the growth regulatory system. Results of Chapter 3 demonstrated a linear increase in the liver Cu concentrations and contents of weaned pigs as sow dietary Cu level increased, which suggests that pigs from sows fed 20 mg/kg Cu may have lower whole body Cu status than those from sows fed 120 mg/kg Cu at the beginning of the present experiment. Logically, one may speculate that when provided with high Cu nursery diets, pigs from sows fed low Cu might exert a greater magnitude of Cu status elevation than those from sows fed high Cu. Consequently, the increased Cu status might enhance synthesis and secretion of growth-related hormones, and eventually exhibit significant growth-promoting effects of high nursery Cu levels within pigs from the sows fed low Cu. Unfortunately, pigs used in the present experiment were not sampled for liver tissues to assess how Cu status changed throughout the experiment.

4.5.2 Lipopolysaccharide challenge

4.5.2.1 Effects of phosphate buffered saline or lipopolysaccharide injection

It was stated by Kusumoto et al. (2010) that a general defense mechanism existed in higher animals to recognize ubiquitous, typical bacterial cell components as alarm signals of infection, and to trigger the host defense system against invading microbes. Lipopolysaccharide is an essential structural element of the outer membrane present in all Gram-negative bacterial cells (Galanos and Freudenberg, 1993). Administration of LPS to animals is extensively used to establish an experimental model of immunological challenge

that will induce a systemic inflammatory response in animal species (Berry et al., 2011; Song et al., 2011; Wang et al., 2011b; Burdick et al., 2012). With regard to pigs, a single injection of LPS can sharply increase the levels of pro-inflammatory cytokines in plasma, activate the hypothalamic-pituitary-adrenal (HPA) axis, and suppress the growth axis (Webel et al., 1997; Liu et al., 2003; Campos et al., 2014); additionally, the LPS injection simultaneously induces depression, loss of appetite, lethargy, and fever of pigs (Sakumoto et al., 2003; Kim et al., 2010).

In the present experiment, pigs injected with LPS had greater BW loss, and less feed intake than pigs injected with PBS during each 2-h period or cumulatively for the first 12 h post-injection. These results agree with previous studies which reported that pigs administrated LPS exerted acute suppression of growth and feed intake during the first 4 to 12 h post-injection (Johnson and von Borell, 1994; Warren et al., 1997; Sakumoto et al., 2003; Kim et al., 2010). In addition, pigs injected with LPS had smaller cumulative BW gain and feed intake for the entire 168 h post-injection period compared to pigs injected with PBS. In agreement, Mao et al. (2005) reported that intramuscular injection of LPS at 200 μg/kg of BW significantly reduced ADG and ADFI of pigs during the first 7 d post-injection when compared to pigs injected with an equal volume of endotoxin-free Hank's Balanced Salt Solution.

All challenged pigs were deprived of feed for 12 h before LPS administration with the purpose of emptying gastrointestinal contents to avoid weight loss through vomiting as well as to enhance appetite and facilitate feed intake. During 0 to 2 h post-injection, the LPS- and PBS-injected pigs had about 150 and 285 g of feed intake, respectively; and their BW change during the same period averaged at -15 and 465 g, respectively. Even though

LPS-injected pigs had a fair amount of feed intake, they still lost BW during the first 2 h post-injection. It was speculated that the BW loss might be because of increased defecation and urination, and could be due to increased protein and lipid degradation that were induced by the LPS injection. It has been concluded that accelerated muscle protein degradation and increased hepatic acute phase protein synthesis are hallmarks of an inflammatory response; in addition, at least 60% of the amino acids used in these acute phase protein synthesis were demonstrated to be from body protein degradation (Johnson, 1997). The pro-inflammatory cytokines play critical roles in the regulation of protein anabolism and catabolism during immunological stress, with interleukin-1 (IL-1) inhibiting anabolic effects of insulin on skeletal muscle (Klasing and Johnstone, 1991), as well as IL-1, IL-6, and TNF-α mediating hepatic acute phase protein synthesis (Ramadori and Christ, 1999). Furthermore, Feingold et al. (1992) reported that intravenous injection of LPS induced greater serum triglycerides levels in rats, and that anti-TNF antibodies and IL-1 receptor antagonist blocked such increase of triglycerides, which indicated that either or both of IL-1 and TNF-α modulated lipid metabolism changes in immunologically stressed animals. In the present experiment, LPS-injected pigs had an abrupt elevation of serum IL-6 and TNF-α level during the first 2 h post-injection, which might suggest tremendous protein and lipid degradation in the body, and consequently caused BW loss.

In addition to acting on periphery targets, pro-inflammatory cytokines exert effects in the central nervous system as well (Johnson and von Borell, 1994). There is evidence that administration of IL-6 and TNF-α through intracerebroventricular injection, which bypasses the blood-brain barrier, induced anorexia, hypersomnia, and an abrupt increase in plasma cortisol concentration of animals (Warren et al., 1997; Wallenius et al., 2002).

These results support the idea that many of the metabolic effects of peripheral immunological stress are mediated by the actions of cytokines in the brain. In the present experiment, serum cortisol levels of LPS-injected pigs were significantly greater than that of PBS-injected pigs over time. Cortisol is secreted in the adrenal cortex within the adrenal gland and regulated by the HPA axis. Increased serum cortisol levels of LPS-injected pigs might indicate that the HPA axis was activated by LPS injection, which is in agreement with previous studies (Webel et al., 1997; Liu et al., 2003; Campos et al., 2014).

In the present experiment, the endotoxin-induced elevation of rectal temperature was consistent with previous studies (Warren et al., 1997; Kim et al., 2010; Moraes et al., 2012). With increasing rectal temperature during LPS challenge, it was expected to observe a higher respiratory rate of LPS-injected pigs. However, the respiratory rate did not differ between LPS- or PBS-injected pigs over time. Kim et al. (2010) and Moraes et al. (2012) reported higher respiratory rate of LPS-injected pigs compared to PBS-injected pigs at specific time points post-injection. However, because the monitoring of respiratory rate is a more subjective measurement that involves the personal judgment of flank movement, the different respiratory rate at specific time points may not suggest a consistent effect throughout the overall challenge period.

4.5.2.2 Effects of sow and nursery copper level

Body weight, feed intake, and vital signs

Since the BW of pigs from sows fed 120 mg/kg Cu tended to be greater ($P = 0.06$) than those from sows fed 20 mg/kg Cu at the end of the growth trial, the BW of LPS-challenged pigs from the 2 sow groups tended to be different before LPS injection ($P = 0.07$), and through the first 12 h post-injection (repeated measures, $P = 0.07$, Table 4.4). Comparing

to BW, the BW change of each period or cumulative periods might be the indexes that are more appropriate to evaluate the response of pigs to LPS administration because they would be less affected by the initial BW. Still, the BW change of each period or cumulative periods were not affected by sow or nursery dietary Cu levels during the challenge period in the current experiment.

Song et al. (2009) fed 1-d old chicks with diets supplemented with 0 or 50 mg/kg Cu as $CuSO_4$ for 24 d, and then intraperitoneally injected with 100 μg/kg of LPS; the results showed that the BW gain and feed intake at 12 or 72 h post-injection were not affected by dietary Cu levels. In addition, Koh et al. (1996) reported that intraperitoneal injection of 300 μg LPS (d 10, 13, and 15 post-hatching) to chicks fed diets supplemented with 0 or 15 mg/kg Cu as $CuSO_4$ from 10 to 16 d post-hatching did not affect ADG ($P = 0.30$), ADFI ($P = 0.14$), or G:F ($P = 0.74$). The results are in agreement with the present experiment, which failed to detect any effect of nursery Cu levels on BW gain and feed intake of post-injected pigs. Klasing and Johnstone (1991) concluded that immunologically stressed animals have a shift in the partitioning of dietary nutrients away from skeletal muscle accretion toward metabolic responses that support the immune system. The lack of difference on BW change of the current experiment might indicate that the LPS-induced repartitioning of nutrients was not affected by sow or nursery dietary Cu levels.

In the present experiment, pigs from sows fed 120 mg/kg Cu had greater rectal temperature (repeated measures, $P = 0.01$) from 0 to 12 h post-injection, as well as greater rectal temperature change from 0 to 2 h post-injection ($P = 0.09$), when compared to those from sows fed 20 mg/kg Cu. Fever is a common manifestation of acute infectious illness and benefits animals by enhancing immune-protective mechanisms during infection (Exton,

1997; Evans et al., 2015). The higher rectal temperature might suggest that the pigs from sows fed high Cu had a greater response to immunological stress as compared to those from sows fed low Cu.

Serum cortisol and cytokines concentrations

It has been reported that intramuscular injection of LPS (75 μg/kg BW) to weanling pigs fed diets supplemented with 250 mg/kg Cu for 13 d resulted in reduced cortisol levels in serum ($P < 0.05$), when compared to pigs fed diets without supplemental Cu (Namkung et al., 2006). In contrast, the present experiment showed that serum cortisol levels of LPS challenged pigs were not affected by sow or nursery dietary Cu levels at each time point or cumulative periods. The discrepancy might be due to the greater BW of pigs at the time of challenge herein [8.5 (Namkunng et al., 2006) vs. 17.8 kg in the present study] or the lower dosage of LPS (75 vs. 50 μg/kg BW of the current experiment). In addition, the greater Cu levels in sow and nursery diets resulted in greater serum cortisol elevation during the 0 to 2 h post-injection period ($P < 0.06$). This suggests that high Cu may induce pigs to have a greater acute response to immunological stress, which agrees with the results that pigs from sows fed high Cu had greater rectal temperature and rectal temperature change during the 0 to 2 h post-injection period compared to sows fed low Cu.

Lipopolysaccharide-induced fever has been reported to not occur in the presence of IL-6-specific neutralizing antibody or IL-6-deficient mice; whereas direct intracerebroventricular injection of IL-6 restores febrile responses in IL-6-deficient mice (Chai et al., 1996; Kozak et al., 1998; Hamzic et al., 2013). These results indicate that IL-6 is an important mediator of fever induction. However, serum IL-6 levels were not

affected by sow or nursery Cu levels throughout the challenge period (repeated measures, $P > 0.44$).

In the present experiment, pigs fed 220 mg/kg Cu diets had greater serum TNF-α levels (repeated measures, $P = 0.03$) than those fed 20 mg/kg Cu diets; and pigs from sows fed 120 mg/kg Cu had numerically greater serum TNF-α levels (repeated measures, $P = 0.13$) than those from sows fed 20 mg/kg Cu. Namkung et al. (2006) reported LPS administration of weanling pigs fed a diet supplemented with 250 mg/kg Cu resulted in numerically lower serum TNF-α levels compared to those fed a diet without Cu supplementation (182 vs. 265 pg/mL). However, the reported TNF-α level of the mentioned study was much lower than those in other studies that collected serum samples at 2 to 4 h post-injection (1500 to 2500 pg/mL) (Sakumoto et al., 2003; Li and Kim, 2013; Weber et al., 2014b).

In addition to animal studies, an in vitro study reported that incubation of macrophages isolated from head kidneys (the portion of the kidney with the largest concentration of immunological cells) of rainbow trout in medium containing Cu and LPS (Cu as copper chloride, 50 μM; LPS, 10 μg/mL) significantly increased TNF-α gene expression compared to those incubated in medium containing LPS only (10 μg/mL) (Teles et al., 2011), which supports the results of the present experiment. Furthermore, when compared to wild-type mice, a mouse line with lung-specific *Tnf-α* overexpression was found to have about 70% reduction of Cu levels in the lung, as well as downregulation of amine oxidase Cu containing 3 and lysyl oxidase, which are Cu dependent enzymes (Liu et al., 2016a). This indicates that Cu may become deficient following a chronic inflammation when TNF-α is tremendously secreted, and higher Cu status or faster Cu restoration may be ideal for animals under chronic inflammatory stress.

4.6 Implication

The results of the present experiment demonstrated that high Cu levels in sow and nursery diets promoted growth performance of nursery pigs interactively, as well as affected responses of pigs to immunological challenge. These results and the literatures reviewed suggest that body Cu status might be important to growth and responses to infection induced immunological stress of pigs, and immunologically stressed animals mobilize a large amount of body Cu reserve. Therefore, future study to assess the bioavailability of different sources of Cu and Cu status of immunologically stressed animals may be needed.

CHAPTER 5. General Discussion

Improving the productivity of sows is one of the approaches to increase pork production because sow productivity determines the number of available pigs for finishing and slaughter. With continuous improvement in genetic selection, nutrition, and management during the past decades, sow productivity has been improved dramatically. However, a conflict exists between the selections for greater productivity and the target of a prolonged productive lifetime. A continuous high culling rate (40 to 50%) and increasing sow death rate (6.5 to 10.0%) have been reported in the US commercial breeding herds during the past 15 years (PigChamp, 2016, 2017). The short productive life of sows is considered harmful to pig production because of more unproductive days, less acquired immunity to herd disease, and greater replacement cost (Lucia et al., 2000b; Hoge and Bates, 2011). The length of productive life of sows is influenced by many factors, which include genetics, management, housing, disease, and nutrition (Farmer, 2015). The modern sows are producing litters of greater number and greater weight, and consequently sows also have to mobilize more nutrients from body reserves to sustain faster fetal development in gestation and greater milk yield in lactation. Mahan and Newton (1995) demonstrated that body mineral contents, which included calcium, phosphorous, magnesium, potassium, sodium, aluminum, zinc, and copper (Cu), were significantly lower in sows that completed three parities compared to those in similarly aged, nongravid gilts. When the mobilization of minerals in body reserve exceeds body stores and dietary intake, reproductive capacity of sows is inevitably compromised (Mahan, 1990).

As one of the minerals that that is being depleted in body storage of sows with advancing parity, Cu is required to serve many biological roles in the body, such as supporting iron

metabolism, protecting tissues from oxidative damage, and maintaining immunity (Mahan and Newton, 1995; Hill and Spears, 2001). Dietary supplementation of pharmacological levels of Cu (250 mg/kg) in gestating and lactating diets from parity 1 to 6 has been demonstrated to increase Cu concentrations in sow liver and kidney, as well as improve piglet weight at birth and weaning; with no detrimental effect on sow longevity (Cromwell et al., 1993). This suggests that high dietary Cu may replenish sow Cu status and improve productivity without compromising longevity.

In the first experiment of the current study (Chapter 3), sows fed high Cu diets (120 and 220 mg/kg) for over 2 yr produced a numerically greater number of litters than sows fed 20 mg/kg Cu (38 and 37 vs. 31 litters; Table 3.4). This demonstrated that feeding high Cu in the long term did not have any negative effect on fertility of sows. This is in agreement with the results of Cromwell et al. (1993), who reported that there was no difference in the number of litters produced by sows fed diets containing 0 or 250 mg/kg of supplemental Cu as $CuSO_4$ for 6 parities.

In the present experiment, increasing dietary Cu levels linearly increased piglet weight of total born ($P = 0.12$) and live born ($P = 0.06$). This was associated with increased liver Cu concentrations of high Cu fed sows (linear, $P < 0.0001$). Fetal development has been demonstrated to be manipulated by IGF-1 through regulating nutrient transfer from mother to fetuses as well as fetal nutrient uptake and metabolism (Gluckman and Pinal, 2003; Bowman et al., 2010) and high dietary Cu levels were reported to increased serum IGF-1 level of growing pigs (Wang et al., 2016).

With regards to weanling pigs, interactions between dietary Cu sources and levels were observed on adjusted piglet weight at weaning ($P = 0.06$) and adjusted piglet weight gain

(P = 0.02), with increasing linear response within TBCC treatments while no response or decreasing linear response within $CuSO_4$ treatments, to the increasing dietary Cu levels. Meanwhile, sows fed TBCC diets had greater ATTD of DM, nitrogen, GE, Ca, and P than those that fed $CuSO_4$ diets during lactation; moreover, milk from sows that fed TBCC diets had greater levels of nutrients than that from sows fed $CuSO_4$ diets, which might support the speculation. These differences between the 2 Cu sources were postulated due to the greater prooxidant activity of $CuSO_4$ than TBCC. The greater oxidative activity might cause an diminished oxidative status of sows, or, alternatively, may damage other nutrients in sow diets during storage which would eventually affect lactation performance of sows and compromise litter performance.

The activity of total and Cu/Zn SOD in sow serum linearly increased with increasing dietary Cu levels regardless of Cu sources (Gestation, $P < 0.10$; Lactation, $P < 0.05$). In piglets at birth, total SOD activity (linear, P = 0.07) and Cu/Zn SOD activity (quadratic, P = 0.09) tended to increase as sow dietary Cu level increased. Copper is a critical constituent of Cu/Zn SOD; it is reversibly oxidized and reduced by successive encounters with O_2^- to yield O_2 and H_2O_2 during SOD catalysis (Tainer et al., 1983). It has been reported that 50 and 250 mg/kg of supplemental Cu as $CuSO_4$ increased serum and erythrocyte SOD activity of growing pigs, respectively, when compared with pigs fed diets without supplemental Cu (Feng et al., 2007; Gonzales-Eguia et al., 2009). Results of the present experiment agree with previous studies.

Liver trace mineral levels are good indicators of body trace mineral status of animals. In the present experiment, sows fed with $CuSO_4$ diets had greater concentration and content of liver Cu than sows fed with TBCC diets ($P < 0.05$). However, liver Fe concentrations of

sows fed TBCC diets were greater than those fed $CuSO_4$ diets in the present experiment ($P < 0.05$). High dietary Cu levels have been demonstrated to depress Fe deposition in liver and kidney of pigs (Hedges and Kornegay, 1973; Bradley et al., 1983). Meanwhile, sows fed with TBCC diets tended to have greater Hb levels during late gestation ($P = 0.10$) and had numerically greater Htc during late gestation and lactation. With regard to their progenies, trace mineral concentrations and contents of neonatal piglets did not differ across treatments. In contrast, liver Cu concentrations and contents of weanling piglets linearly increased as sow dietary Cu level increased ($P < 0.05$). It might be associated with Cu concentrations in milk, which exerted a significant linear increase with increasing sow dietary Cu levels.

In summary, results of the first experiment indicated that TBCC might be a superior Cu source compared to $CuSO_4$ regarding reproductive performance; the feeding of a high Cu diet did not have any apparent negative effects on sows and progenies but resulted in a heavier birth weight and greater liver Cu levels at weaning.

With the realization of the fact that piglets from sows fed high Cu had a greater amount of Cu deposited in the liver at weaning compared to those from sows fed low Cu, the question arose whether the greater Cu status would affect growth performance and health of weanling pigs during the subsequent nursery phase. Therefore, the effects of dietary Cu levels on growth performance, and response to a lipopolysaccharide challenge of nursery pigs from sows fed with high or low Cu diets were assessed in the second experiment (Chapter 4).

In the growth phase of the second experiment, an interaction between maternal and nursery Cu levels on d 14 to 21 ADG ($P = 0.04$) demonstrated that a high nursery dietary

Cu level was effective in improving the growth rate of pigs from 20 mg/kg Cu fed sows but not for those from sows fed 120 mg/kg Cu. It was speculated that the interactive effects were due to the different Cu status of pigs at the beginning of the trial. The ones with lower initial Cu status may have a faster pace and greater magnitude of Cu status elevation and may have greater stimulation of the GH axis, and then eventually exhibit significant growth-promoting effects of the higher nursery Cu levels.

During the LPS challenge period, pigs from sows fed 120 mg/kg Cu had a greater overall rectal temperature than those from sows fed 20 mg/kg Cu ($P = 0.01$). Also, the challenged pigs fed with 220 mg/kg Cu diets had greater TNF-α levels over time compared to those fed 20 mg/kg Cu diets ($P = 0.03$). It has been demonstrated in chicks and mice that body reserves of Cu are greatly mobilized during immunological stress (Klasing and Barnes, 1988; Han et al., 2013), and chronic inflammation might cause Cu deficiency in lung tissue of mice (Liu et al., 2016a). These previous studies indicate that Cu is required to support the responses associated with infectious inflammation. Results of the present experiment also suggest that greater body Cu status or Cu supply in diets are associated with greater response to immunological stress in pigs.

In summation, many positive efffects were observed to an enhanced Cu status of sows and nursery pigs. Therefore, further studies to assess the effects of supplementing organic sources of Cu in sow diets with reduced levels on reproductive performance and health of sows; or to assess the bioavailability of different sources of Cu on Cu status of immunologically stressed animals may be warranted. Dose response studies to define Cu supplementation levels above the current NRC (2012) requirement estimates that will

maximize immune response would also seem to have value for improved swine health and wellbeing.

APPENDICES

Appendix 1. Analyzed trace mineral concentrations of gestation and lactation diets throughout the fecal collection period

Table A.1.1. Analyzed trace mineral concentrations in gestation diets throughout the fecal collection period

	Batch											
	2014		2015									
Item	Aug	Dec	Jan	Feb	Mar	Apr	May	Jun	Jul	Sep	Oct	Dec
Diet 1, 20 mg/kg copper as tribasic copper chloride												
Copper	18			24			24		27		31	
Iron	313			405			290		359		340	
Manganese	84			82			82		134		120	
Zinc	183			184			155		200		186	
Diet 2, 120 mg/kg copper as tribasic copper chloride												
Copper	100		101		110			107	90		109	
Iron	318		330		328			344	264		302	
Manganese	77		78		71			79	72		76	
Zinc	170		172		175			154	155		161	
Diet 3, 220 mg/kg copper as tribasic copper chloride												
Copper	225	235		235		201			196	222		
Iron	366	399		420		323			343	343		
Manganese	74	77		73		77			76	79		
Zinc	176	179		176		151			157	157		

Continued

Table A.1.1 continued

Diet 4, 20 mg/kg copper as copper sulfate							
Copper	31	27	28		36		
Iron	321	440	377		360		
Manganese	81	78	89		95		
Zinc	170	181	167		177		
Diet 5, 120 mg/kg copper as copper sulfate							
Copper		134	195	133	170	135	196
Iron		371	411	337	371	380	406
Manganese		87	84	82	98	86	93
Zinc		158	166	149	172	155	151
Diet 6, 220 mg/kg copper as copper sulfate							
Copper	251	203	275	215	335	264	
Iron	369	373	472	347	406	380	
Manganese	82	82	94	79	98	84	
Zinc	159	161	171	150	165	213	

Table A.1.2. Analyzed trace mineral concentrations in lactation diets throughout the fecal collection period

	Batch									
	2014			2015						
Item	Sep	Oct	Nov	Feb	Mar	Jul	Aug	Sep	Oct	Dec
Diet 1, 20 mg/kg copper as tribasic copper chloride										
Copper	25				28	27	27			26
Iron	381				447	342	343			333
Manganese	90				99	120	115			110
Zinc	189				186	188	181			187
Diet 2, 120 mg/kg copper as tribasic copper chloride										
Copper	126	133		102	103	113		126		124
Iron	322	217		451	394	347		411		365
Manganese	86	82		87	83	77		84		82
Zinc	174	178		174	171	162		168		162
Diet 3, 220 mg/kg copper as tribasic copper chloride[1]										
Copper	220			228	215 (206)		227		221	
Iron	270			363	391 (391)		338		399	
Manganese	74			77	80 (72)		80		79	
Zinc	168			173	165 (156)		165		158	
Diet 4, 20 mg/kg copper as copper sulfate										
Copper	35			38	33		32	32		
Iron	339			375	397		384	388		
Manganese	92			90	97		82	92		
Zinc	167			173	161		155	153		

Continued

Table A.1.2 continued

Diet 5, 120 mg/kg copper as copper sulfate					
Copper		137	132	129	131	138
Iron		410	342	351	377	323
Manganese		106	94	84	97	80
Zinc		168	160	149	152	146
Diet 6, 220 mg/kg copper as copper sulfate[1]					
Copper	277		246 (239)		256	
Iron	348		353 (414)		332	
Manganese	94		91 (84)		86	
Zinc	172		158 (148)		156	

[1]Numbers in parentheses represent the analyzed trace mineral concentrations of another mixing batch during the same month.

Appendix 2. Numbers of excluded outliers in Chapter 3

Table A.2.1. Number of excluded litters and individual observations of sow and litter performance, and apparent total tract digestibility in Chapter 3[1, 2]

Copper source:	Tribasic copper chloride			Copper sulfate		
Copper level, mg/kg:	20	120	220	20	120	220
Item Diet No.:	1	2	3	4	5	6
Litter excluded						
No piglet alive at weaning[3]	1 (1)	0	0	1 (1)	0	2 (2)
Less than 5 piglets born alive[3]	0	3 (1 and 3)	4 (1, 2, and 4)	0	2 (1)	1 (1)
At least 3 piglets were crushed to death[3]	2 (2 and 4)	0	0	0	0	3 (2, 3, and 4)
Individual observations excluded						
Sow weight change						
Late gestation to post farrowing	3 (1, 3, and 4)	0	0	0	1 (3)	2 (1 and 4)
Breeding to late gestation	0	0	2 (3)	1 (3)	1 (2)	2 (3 and 4)
Litter size[4]	1 (4)	0	1 (2)	0	0	1 (4)
Apparent total tract digestibility						
Late gestation sow						
Ether extract	1 (3)	2 (3)	2 (2 and 3)	2 (3)	1 (3)	6 (2)
Calcium	0	1 (3)	0	0	0	0
Phosphorous	0	0	0	0	0	1 (3)
Copper	1 (2)	0	0	1 (2)	0	1 (2)
Iron	0	0	0	0	0	1 (3)

Continued

Table A.2.1 continued

Manganese	0	1 (2)	0	0	0	0
Lactating sow						
Dry matter	0	0	1 (2)	0	0	0
Phosphorous	0	0	1 (2)	0	0	0

[1]Numbers in parentheses represent the parity of sows for the excluded litters or observations.
[2]Excluded individual observations were detected by Grubb's test outlier calculator.
[3]Data of the entire litter was excluded because these litters were considered to be qualitatively different from other litters.
[4]Litter size includes the observations of numbers of piglets total born, live born, stillborn, and weaning, as well as mortality and survival rate.

Table A.2.2. Number of excluded individual observations of serum, colostrum, and milk measurements in Chapter 3[1, 2]

Copper source:	Tribasic copper chloride			Copper sulfate		
Copper level, mg/kg:	20	120	220	20	120	220
Item Diet No.:	1	2	3	4	5	6
Serum						
Late gestation sow						
Hemoglobin	0	1 (2)	0	0	0	0
Superoxide dismutase	0	0	1 (2)	0	1 (2)	0
Ceruloplasmin	0	1 (3)	0	0	0	0
Lactating sow						
Hemoglobin	0	0	0	0	1 (3)	1 (2)
Hematocrit	0	0	0	0	0	1 (2)
Superoxide dismutase	0	0	1 (2)	0	0	1 (2)
Ceruloplasmin	0	1 (4)	0	1 (2)	0	0
Malondialdehyde	0	1 (3)	1 (3)	0	0	1 (3)
Piglet at weaning						
Hemoglobin	1 (3)	0	0	0	0	0
Hematocrit	1 (3)	0	2 (3)	0	0	0
Superoxide dismutase	0	0	0	1 (3)	1 (3)	0
Ceruloplasmin	0	0	1 (3)	0	0	0
Malondialdehyde	0	1 (4)	0	0	0	0
Colostrum and milk						
Colostrum						
Superoxide dismutase	1 (3)	0	0	0	0	0
Fat	1 (3)	0	0	0	0	0
Lactose	0	0	0	1 (4)	0	0
Iron	0	0	1 (3)	0	0	0
Milk						
Superoxide dismutase	0	1 (3)	0	0	0	0
Protein	0	1 (3)	0	0	0	0
Lactose	0	1 (3)	0	0	0	0
Copper	0	0	0	1 (3)	0	0

[1]Number in parentheses represents the parity of sows for the excluded observation.
[2]Excluded individual observations were detected by Grubb's test outlier calculator.

Table A.2.3. Number of excluded individual observations of tissue trace mineral measurements in Chapter 3[1, 2]

Copper source:	Tribasic copper chloride			Copper sulfate		
Copper level, mg/kg:	20	120	220	20	120	220
Item Diet No.:	1	2	3	4	5	6
Sow						
Liver						
Copper	0	1 (3)	0	0	0	0
Iron	0	0	0	0	0	1 (4)
Heart						
Copper	0	1 (3)	0	0	0	0
Iron	0	1 (3)	0	0	0	0
Manganese	0	1 (3)	0	0	0	0
Zinc	0	1 (3)	0	0	0	0
Piglets at birth						
Heart						
Iron	0	0	0	0	1 (3)	0
Zinc	0	1 (3)	0	0	0	0
Kidney						
Iron	0	0	1 (4)	0	0	0
Piglet at weaning						
Liver						
Iron	0	0	0	0	1 (4)	1 (4)

[1]Number in parentheses represents the parity of sows for the excluded observation.
[2]Excluded individual observations were detected by Grubb's test outlier calculator.

Appendix 3. Post hoc statistical power analysis of reproductive performance data

Table A.3.1. Post hoc statistical power analysis of reproductive performance data from parity 1 to 4 sows (88 litters)

	Main effects:	Source[1]			Level[2]			Source × Level[3]		
Item	Significance level:	0.05	0.10	Increase, %[4]	0.05	0.10	Increase, %[4]	0.05	0.10	Increase, %[4]
Sow weight										
Breeding		0.34	0.46	37	0.08	0.15	81	0.09	0.16	79
Late gestation		0.27	0.39	44	0.15	0.25	60	0.07	0.14	86
Post farrowing		0.27	0.39	43	0.38	0.51	34	0.08	0.15	82
Weaning		0.23	0.34	47	0.36	0.49	35	0.18	0.29	57
Sow weight changes										
Breeding to late gestation		0.13	0.21	63	0.15	0.24	61	0.12	0.21	69
Late gestation to post farrowing		0.06	0.11	95	0.32	0.45	39	0.05	0.10	100
Post farrowing to weaning		0.05	0.10	100	0.07	0.12	89	0.10	0.17	77
Late gestation to weaning		0.05	0.10	99	0.13	0.22	65	0.08	0.15	82
Lactation daily feed intake		0.48	0.60	27	0.08	0.14	82	0.21	0.33	52
Litter size										
Total born		0.39	0.52	33	0.11	0.18	72	0.12	0.21	69
Live born		0.11	0.19	68	0.06	0.11	96	0.08	0.15	82
Still born		0.71	0.81	14	0.25	0.36	46	0.13	0.22	66
Weaning		0.25	0.37	44	0.07	0.13	87	0.07	0.14	86
Mortality		0.29	0.41	41	0.08	0.15	81	0.07	0.13	89
Survival rate		0.44	0.57	29	0.07	0.13	85	0.05	0.10	98

Continued

Table A.3.1 continued

Litter weight									
Total born	0.69	0.79	15	0.18	0.29	55	0.05	0.10	98
Live born	0.41	0.54	31	0.13	0.21	66	0.06	0.11	94
Weaning	0.67	0.78	16	0.10	0.17	74	0.13	0.22	67
Litter gain	0.70	0.80	15	0.17	0.26	58	0.19	0.30	56
Piglet weight									
Total born	0.19	0.29	52	0.45	0.58	29	0.09	0.16	80
Live born	0.31	0.43	39	0.50	0.63	26	0.11	0.18	74
Weaning	0.53	0.65	23	0.08	0.15	80	0.39	0.52	34
Piglet gain	0.54	0.67	23	0.06	0.12	92	0.45	0.58	30
Adjusted weight									
Litter weight at weaning	0.74	0.83	13	0.06	0.12	92	0.17	0.27	59
Piglet weight at weaning	0.61	0.72	19	0.19	0.30	54	0.55	0.67	23
Litter weight gain at weaning	0.80	0.87	10	0.08	0.15	79	0.28	0.41	44
Piglet weight gain at weaning	0.65	0.76	17	0.11	0.18	71	0.68	0.79	16

[1]Tribasic copper chloride = 43 litters; copper sulfate = 45 litters.
[2]Copper levels at: 20 mg/kg = 28 litters; 120 mg/kg = 33 litters; 220 mg/kg = 27 litters.
[3]Diet 1 = 11 litters; diet 2 = 19 litters; diet 3 = 15 litters; diet 4 = 17 litters; diet 5 = 14 litters; diet 6 = 12 litters.
[4]Percentage unit increase of statistical power with significance level at 0.10 as compared to at 0.05.

Appendix 4. Effects of parity on reproductive performance of sows

Table A.4.1. Effects of parity on reproductive performance of sows

Item	Parity				SEM	Parity 1 vs. 2 to 4[1]	*P* values	
	1	2	3	4			L	Q
No. of litters	21	25	24	18				
Sow weight, kg								
Breeding	177.3	201.4	210.0	214.2	4.2	< 0.0001	< 0.0001	0.02
Late gestation	222.5	239.8	233.1	236.4	4.1	0.006	0.08	0.10
Post farrowing	204.6	223.2	216.8	221.6	4.0	0.001	0.02	0.09
Weaning	197.2	228.8	236.1	235.6	4.1	< 0.0001	< 0.0001	0.0002
Sow weight changes, kg								
Breeding to late gestation	46.38	39.68	25.10	26.84	2.5	< 0.0001	< 0.0001	0.10
Late gestation to post farrowing	-19.88	-18.60	-18.24	-16.83	1.7	0.36	0.23	0.97
Post farrowing to weaning	-11.37	1.46	15.44	10.33	2.4	< 0.0001	< 0.0001	0.0004
Late gestation to weaning	-31.25	-17.14	-2.81	-6.50	3.0	< 0.0001	< 0.0001	0.003
Lactation daily feed intake, kg/d	4.21	5.86	6.14	5.82	0.17	< 0.0001	< 0.0001	< 0.0001
Litter size								
Total born	8.52	9.96	10.71	11.33	0.49	0.0006	0.0002	0.42
Live born	7.86	9.48	10.17	10.17	0.48	0.0003	0.001	0.10
Still born	0.67	0.44	0.54	1.17	0.21	0.85	0.11	0.05
Weaning	7.57	8.80	8.58	8.50	0.45	0.02	0.22	0.146
Mortality	0.29	0.68	0.63	0.67	0.16	0.10	0.16	0.29

Continued

Table A.4.1 continued

Survival rate, %[2]	96.17	92.83	93.66	93.71	1.76	0.30	0.43	0.34
Litter weight, kg								
Total born	13.37	16.02	16.30	16.56	0.79	0.002	0.009	0.13
Live born	12.55	15.43	15.49	15.26	0.78	0.003	0.03	0.05
Weaning	47.41	59.37	54.68	51.20	2.81	0.03	0.61	0.008
Litter gain[2]	35.13	44.86	41.33	38.24	2.23	0.02	0.05	0.01
Piglet weight, kg								
Total born	1.59	1.63	1.54	1.46	0.06	0.57	0.10	0.31
Live born	1.61	1.66	1.55	1.49	0.06	0.57	0.10	0.37
Weaning	6.41	6.83	6.46	6.03	0.23	0.86	0.17	0.07
Piglet gain[2]	6.41	6.83	6.46	6.03	0.23	0.68	0.25	0.06
Adjusted weight, kg								
Litter weight at weaning	48.89	58.78	56.07	53.01	2.87	0.04	0.47	0.03
Piglet weight at weaning	6.62	6.87	6.57	6.25	0.23	0.84	0.19	0.22
Litter weight gain[2]	36.67	44.43	42.63	39.75	2.16	0.03	0.46	0.02
Piglet weight gain[2]	4.97	5.20	4.99	4.70	0.18	0.96	0.23	0.16

[1]A single degree of freedom contrast was conducted to compare the difference between litters of the first parity and the other parities.
[2]Because 1 piglet was sacrificed at birth for the third and fourth litters, adjusted live born litter size or litter weight were used in the calculations of survival rate, litter gain, and piglet gain. The adjustment was applied through excluding the sacrificed piglet.

Appendix 5. Effects of dietary copper sources and levels on reproductive performance of gilts or pooled gilts and sows

Table A.5.1. Effects of dietary copper sources and levels on reproductive performance of gilts

Copper source:	Tribasic copper chloride			Copper sulfate					
Copper level, mg/kg:	20	120	220	20	120	220		*P* values	
Item Diet No.:	1	2	3	4	5	6	SEM	Source	Level
No. of litters	3	4	3	4	3	4			
Sow weight, kg									
Breeding	182.0	188.0	182.3	165.8	181.1	167.8	12.2	0.23	0.64
Late gestation	215.0	231.5	223.7	217.8	226.3	216.3	11.9	0.74	0.56
Post farrowing	197.0	211.3	211.7	194.3	212.6	199.7	10.5	0.61	0.33
Weaning	188.7	206.5	200.5	183.1	208.8	194.3	15.1	0.80	0.39
Sow weight changes, kg									
Breeding to late gestation	49.4	43.4	41.6	52.2	45.5	46.3	4.3	0.39	0.27
Late gestation to post farrowing	-20.4	-22.3	-14.1	-25.5	-16.1	-18.5	3.6	0.72	0.25
Post farrowing to weaning	-14.2	-7.8	-14.2	-14.1	-10.6	-8.6	9.4	0.91	0.87
Late gestation to weaning	-34.5	-30.3	-28.3	-39.6	-26.7	-27.0	9.6	0.99	0.60
Lactation daily feed intake, kg/d	4.67	4.50	4.00	3.63	4.00	4.25	0.67	0.44	0.98
Litter size									
Total born	9.33	10.25	7.67	8.75	7.00	7.75	1.23	0.23	0.55
Live born	7.67	9.75	7.67	7.25	7.00	7.50	1.08	0.23	0.66
Still born[1]	1.67	0.50	0.00	1.50	0.00	0.25	0.47	0.72	0.01
Weaning	7.33	9.50	7.67	6.75	6.67	7.25	1.08	0.17	0.64

Continued

Table A.5.1 continued

Mortality	0.33	0.25	0.00	0.50	0.33	0.25	0.27	0.47	0.57
Survival rate, %[2]	95.83	97.73	100.00	90.83	96.30	97.22	3.63	0.32	0.36
Litter weight, kg									
Total born	13.75	16.12	13.54	13.43	10.88	12.04	2.07	0.19	0.92
Live born	12.06	15.43	13.54	11.45	10.88	11.64	1.86	0.14	0.76
Weaning	48.00	56.14	54.46	42.57	39.32	43.87	6.29	0.05	0.83
Litter gain[2]	36.31	40.87	40.92	31.29	29.08	32.56	4.85	0.05	0.83
Piglet weight, kg									
Total born	1.48	1.64	1.77	1.53	1.59	1.54	0.16	0.57	0.65
Live born	1.58	1.64	1.77	1.57	1.59	1.54	0.16	0.47	0.89
Weaning	6.56	6.20	7.33	6.39	6.12	6.08	0.74	0.42	0.76
Piglet gain[2]	4.97	4.53	5.55	4.68	4.55	4.53	0.63	0.41	0.74
Adjusted weight, kg									
Litter weight at weaning	49.41	58.36	54.63	43.36	41.50	46.09	6.22	0.06	0.79
Piglet weight at weaning	6.72	6.38	7.30	6.57	6.37	6.37	0.62	0.49	0.76
Litter weight gain[2]	37.72	43.09	41.08	32.08	31.25	34.78	4.64	0.05	0.80
Piglet weight gain[2]	5.14	4.71	5.52	4.86	4.80	4.82	0.51	0.49	0.72

[1]Linear response to copper level ($P < 0.10$).

[2]Because 1 piglet was sacrificed at birth for the thrid and fourth litters, adjusted live born litter size or litter weight were used in the calculations of survival rate, litter gain, and piglet gain. The adjustment was applied through excluding the sacrificed piglet.

Table A.5.2. Effects of dietary copper sources and levels on reproductive performance of pooled gilts and sows

Copper source:	Tribasic copper chloride			Copper sulfate					
Copper level, mg/kg:	20	120	220	20	120	220		*P* values	
Item Diet No.:	1	2	3	4	5	6	SEM	Source	Level
No. of litters	11	19	15	17	14	12			
Sow weight, kg									
Breeding[2]	202.0	202.0	205.8	195.1	200.7	195.9	5.5	0.19	0.87
Late gestation[2]	230.2	235.9	239.7	228.0	231.0	230.0	5.9	0.25	0.64
Post farrowing[2]	211.2	220.1	224.4	209.5	215.7	214.5	5.6	0.26	0.28
Weaning[2]	221.2	226.1	231.9	216.0	228.8	219.7	5.7	0.30	0.29
Sow weight changes, kg									
Breeding to late gestation[2]	37.3	33.9	33.8	34.3	32.3	38.2	3.7	0.98	0.65
Late gestation to post farrowing	-20.9	-17.9	-17.5	-20.4	-17.4	-17.4	2.3	0.85	0.34
Post farrowing to weaning[2]	3.9	3.1	4.6	3.5	6.1	2.1	3.4	0.99	0.93
Late gestation to weaning[2]	-16.9	-14.9	-12.8	-16.9	-11.4	-15.3	4.3	0.93	0.68
Lactation daily feed intake, kg/d[2]	5.50	5.49	5.82	5.27	5.49	5.15	0.23	0.11	0.88
Litter size									
Total born[2]	10.33	10.55	10.55	10.39	9.26	9.31	0.69	0.16	0.78
Live born[2]	9.42	9.76	9.68	9.66	9.08	9.08	0.68	0.54	0.97
Still born	0.83	0.73	0.87	0.73	0.25	0.23	0.23	0.04	0.44
Weaning	8.67	8.81	8.73	8.56	8.00	8.02	0.59	0.27	0.92
Mortality	0.25	0.45	0.45	0.60	0.57	0.65	0.22	0.23	0.86
Survival rate, %[4]	96.99	95.87	95.90	94.00	93.75	93.09	2.06	0.13	0.90

Continued

Table A.5.2 continued

Litter weight, kg									
Total born[2]	16.27	16.00	17.27	14.50	14.40	15.26	1.04	0.04	0.56
Live born[2]	15.21	14.99	15.96	13.60	14.15	14.56	1.05	0.14	0.70
Weaning[2]	55.79	55.51	57.62	53.41	49.59	46.71	3.75	0.04	0.79
Litter gain[2, 4]	42.03	41.72	43.30	40.97	36.86	33.83	2.93	0.04	0.60
Piglet weight, kg									
Total born[3]	1.52	1.56	1.69	1.44	1.57	1.58	0.08	0.36	0.20
Live born[3]	1.55	1.58	1.75	1.46	1.57	1.59	0.08	0.21	0.16
Weaning	6.44	6.41	7.15	6.36	6.34	5.93	0.32	0.08	0.85
Piglet gain[1, 4]	4.85	4.82	5.38	4.85	4.74	4.31	0.26	0.08	0.95
Adjusted weight, kg									
Litter weight at weaning[2]	55.70	57.04	59.51	53.98	50.63	48.28	3.50	0.03	0.95
Piglet weight at weaning[1]	6.44	6.58	7.38	6.47	6.46	6.14	0.28	0.06	0.54
Litter weight gain[2, 4]	41.95	43.25	45.19	41.54	37.89	35.40	2.63	0.02	0.85
Piglet weight gain[1, 4]	4.86	4.99	5.61	4.97	4.86	4.51	0.22	0.05	0.76

[1]Significant copper source × copper level interaction ($P < 0.10$).
[2]Significant sow parity effects ($P < 0.10$).
[3]Linear response to copper level ($P < 0.10$).
[4]Because 1 piglet was sacrificed at birth for the third and fourth litters, adjusted live born litter size or litter weight were used in the calculations of survival rate, litter gain, and piglet gain. The adjustment was applied through excluding the sacrificed piglet.

Appendix 6. Effects of lipopolysaccharide (LPS) or phosphate-buffered saline (PBS) administration on 20 or 220 mg/kg copper fed nursery pigs that from 20 or 120 mg/kg copper fed sows.

Table A.6.1. Effects of sow and nursery dietary copper levels (mg/kg) on response of body weight to LPS or PBS injection

	Challenge:	LPS				PBS					
	Sow copper:	20	20	120	120	20	20	120	120		
	Nursery copper:	20	220	20	220	20	220	20	220		*P* values
Item	Treatment No.:	1	2	3	4	1	2	3	4	SEM	Challenge
No. of observations		4	4	4	4	4	4	4	4		
Body weight, kg											
0 h		16.64	17.25	18.94	18.36	17.22	18.45	18.42	18.98	1.13	0.43
2 h		16.73	17.19	18.90	18.31	17.69	19.02	18.86	19.36	1.17	0.14
4 h		16.61	17.17	18.81	18.14	17.87	19.14	19.08	19.64	1.19	0.06
6 h		16.60	17.18	18.84	18.05	18.00	19.34	19.22	19.67	1.21	0.04
8 h		16.63	17.29	18.96	18.03	18.26	19.48	19.27	19.73	1.23	0.03
10 h		16.62	17.30	18.92	18.09	18.47	19.55	19.35	19.76	1.21	0.03
12 h		16.62	17.23	18.88	18.01	18.45	19.59	19.33	19.77	1.20	0.02
24 h		16.94	17.28	18.95	18.15	18.49	19.90	19.44	20.03	1.25	0.02
48 h		17.51	18.20	19.67	18.86	19.26	20.32	20.11	20.69	1.32	0.03
72 h		18.07	18.85	20.09	19.57	19.74	21.22	20.65	21.31	1.31	0.03
96 h		18.55	19.49	20.48	20.07	20.66	22.06	21.38	22.19	1.34	0.02
120 h		19.31	20.27	21.23	20.75	21.50	22.71	22.39	22.97	1.34	0.01
144 h		20.30	21.30	22.42	21.60	22.63	23.66	23.60	24.10	1.43	0.02
168 h		20.70	21.54	22.81	22.15	23.13	24.14	24.11	24.74	1.48	0.01

Table A.6.2. Effects of sow and nursery dietary copper levels (mg/kg) on response of body weight changes to LPS or PBS injection

	Challenge:	LPS				PBS					
	Sow copper:	20	20	120	120	20	20	120	120		
	Nursery copper:	20	220	20	220	20	220	20	220		*P* values
Item	Treatment No.:	1	2	3	4	1	2	3	4	SEM	Challenge
No. of observations		4	4	4	4	4	4	4	4		
Body weight change, g											
0 to 2 h		88	-52	-45	-50	466	570	444	379	112	< 0.0001
2 to 4 h		-122	-23	-92	-167	210	118	213	279	61	< 0.0001
4 to 6 h		-12	8	28	-96	139	200	150	33	71	0.01
6 to 8 h		29	115	120	-15	257	141	49	61	78	0.26
8 to 10 h		-10	9	-41	56	188	68	75	34	86	0.14
10 to 12 h		1	-75	-35	-78	-39	41	-23	2	68	0.40
12 to 24 h		319	58	69	143	41	315	110	265	226	0.78
24 to 48 h		575	916	717	711	840	418	673	660	237	0.60
48 to 72 h[1]		558	645	429	706	491	892	542	617	154	0.58
72 to 96 h		477	642	383	505	908	841	734	882	143	0.002
96 to 120 h		762	780	755	676	813	656	1001	780	103	0.35
120 to 144 h		991	1034	1186	848	1145	942	1218	1133	142	0.27
144 to 168 h2		398	243	387	550	491	483	505	641	169	0.27

Continued

Table A.6.2 continued

Cumulative weight change, g										
0 to 2 h	88	-52	-45	-50	466	570	444	379	112	< 0.0001
0 to 4 h	-34	-75	-137	-217	676	688	658	658	157	< 0.0001
0 to 6 h	-46	-67	-109	-313	818	888	807	690	205	< 0.0001
0 to 8 h	-17	48	11	-328	1075	1028	856	752	219	< 0.0001
0 to 10 h	-27	57	-29	-272	1237	1096	931	786	245	< 0.0001
0 to 12 h	-26	-18	-65	-350	1197	1137	908	788	265	< 0.0001
0 to 24 h	293	40	5	-207	1242	1452	1018	1053	350	0.0001
0 to 48 h	867	956	721	503	2049	1871	1692	1713	510	0.01
0 to 72 h	1425	1601	1150	1210	2564	2763	2234	2330	506	0.004
0 to 96 h	1902	2243	1533	1714	3453	3604	2967	3212	518	0.001
0 to 120 h	2664	3023	2288	2390	4260	4261	3968	3992	525	0.001
0 to 144 h	3655	4057	3474	3238	5445	5203	5186	5125	595	0.001
0 to 168 h	4053	4299	3861	3788	5922	5686	5690	5765	632	0.001

[1]Nursery dietary copper effect (P = 0.03).

Table A.6.3. Effects of sow and nursery dietary copper levels (mg/kg) on response of feed intake to LPS or PBS injection

Challenge:	LPS				PBS					
Sow copper:	20	20	120	120	20	20	120	120		
Nursery copper:	20	220	20	220	20	220	20	220		*P* values
Item Treatment No.:	1	2	3	4	1	2	3	4	SEM	Challenge
No. of observations	4	4	4	4	4	4	4	4		
Feed intake, g										
0 to 2 h	146	192	155	108	266	319	293	260	49	0.00
2 to 4 h	18	12	1	12	128	101	94	134	27	< 0.0001
4 to 6 h	18	27	5	2	137	133	127	87	23	< 0.0001
6 to 8 h	28	25	1	1	106	78	79	62	23	0.00
8 to 10 h[1]	46	19	8	0	127	77	118	57	22	0.00
10 to 12 h	45	9	7	1	33	93	79	63	26	0.01
12 to 24 h	252	143	137	136	399	432	422	510	106	0.00
24 to 48 h	693	825	546	635	1132	922	857	905	190	0.04
48 to 72 h	683	791	545	677	947	879	795	795	111	0.02
72 to 96 h	884	995	667	747	1190	1138	1007	1151	111	0.00
96 to 120 h	1069	1107	880	873	1285	1160	1266	1169	114	0.01
120 to 144 h	1390	1463	1387	1249	1626	1506	1613	1522	145	0.04
144 to 168 h	931	864	756	1683	994	1008	1116	1000	344	0.90
Cumulative feed intake,										
0 to 2 h	146	192	155	108	266	319	293	260	49	0.0013
0 to 4 h	164	204	156	120	395	420	387	393	69	0.0002

Continued

Table A.6.3 continued

0 to 6 h	183	231	161	122	532	552	514	481	87	< 0.0001
0 to 8 h	211	256	162	124	638	630	593	543	94	< 0.0001
0 to 10 h	257	276	170	124	765	707	711	600	103	< 0.0001
0 to 12 h	303	285	177	125	798	800	790	663	109	< 0.0001
0 to 24 h	554	427	314	261	1187	1232	1212	1173	178	< 0.0001
0 to 48 h	1247	1253	861	896	2295	2154	2069	2078	340	0.0003
0 to 72 h	1930	2044	1406	1573	3256	3033	2864	2873	426	0.0005
0 to 96 h	2814	3040	2073	2320	4441	4171	3871	4024	505	0.0004
0 to 120 h	3883	4146	2952	3193	5712	5331	5137	5193	591	0.0004
0 to 144 h	5273	5609	4339	4442	7347	6837	6751	6714	699	0.0006
0 to 168 h	6204	6473	4728	6125	8256	7845	7866	7714	888	0.002

[1]Nursery dietary copper effects (P = 0.03).

Table A.6.4. Effects of sow and nursery dietary copper levels (mg/kg) on response of rectal temperature to LPS or PBS injection

	Challenge:	LPS				PBS					
	Sow copper:	20	20	120	120	20	20	120	120		
	Nursery copper:	20	220	20	220	20	220	20	220		*P* values
Item	Treatment No.:	1	2	3	4	1	2	3	4	SEM	Challenge
No. of observations		4	4	4	4	4	4	4	4		
Rectal temperature, °C											
0 h[3]		39.04	39.14	39.81	39.28	38.81	39.13	39.38	39.14	0.20	0.16
2 h[1, 3]		40.36	40.13	41.21	40.76	39.28	39.93	40.06	39.79	0.19	< 0.0001
4 h[1, 3]		40.45	40.38	41.17	40.81	39.57	40.02	40.13	39.77	0.17	< 0.0001
6 h		40.33	40.32	40.60	40.74	39.55	40.07	40.06	39.86	0.19	0.0001
8 h		40.20	39.96	40.09	40.57	39.43	39.92	40.07	39.89	0.20	0.01
10 h		40.09	40.03	40.22	40.53	39.58	39.79	40.10	39.89	0.18	0.01
12 h		40.03	39.95	40.32	40.29	39.62	39.79	40.00	39.89	0.21	0.04
Rectal temperature change, °C											
0 to 2 h		1.32	0.99	1.40	1.49	0.48	0.81	0.68	0.65	0.17	< 0.0001
2 to 4 h		0.09	0.25	-0.04	0.04	0.31	0.08	0.07	-0.03	0.14	0.80
4 to 6 h[2]		-0.11	-0.05	-0.57	-0.07	0.00	0.06	-0.07	0.10	0.11	0.01
6 to 8 h		-0.14	-0.36	-0.51	-0.17	-0.15	-0.15	0.01	0.03	0.11	0.01
8 to 10 h		-0.11	0.07	0.14	-0.04	0.15	-0.13	0.03	0.00	0.10	0.99
10 to 12 h		-0.05	-0.08	0.10	-0.24	0.04	0.00	-0.10	0.00	0.10	0.45

[1]Sow dietary copper effects ($P < 0.05$).
[2]Nursery dietary copper effects ($P = 0.02$).
[3]Sow dietary copper × nursery dietary copper interaction ($P < 0.05$).

Table A.6.5. Effects of sow and nursery dietary copper levels (mg/kg) on response of respiratory rate to LPS or PBS injection

Challenge:	LPS				PBS					
Sow copper:	20	20	120	120	20	20	120	120		
Nursery copper:	20	220	20	220	20	220	20	220		*P* values
Item Treatment No.:	1	2	3	4	1	2	3	4	SEM	Challenge
No. of observations	4	4	4	4	4	4	4	4		
Respiratory rate, breath/min										
-0.5 h	39.38	40.13	49.25	50.13	38.82	47.63	53.13	46.25	4.02	0.53
1 h	52.88	70.63	66.88	76.13	66.00	53.88	67.25	52.50	13.25	0.48
3 h	45.00	53.88	51.25	63.13	88.83	79.88	51.63	66.38	8.50	0.01
5 h	58.75	51.75	70.38	68.50	70.05	71.13	74.38	68.25	11.44	0.30
7 h	59.88	52.88	58.75	65.00	69.74	66.75	67.00	54.38	9.44	0.41
9 h	53.75	56.38	49.88	77.38	52.17	77.38	65.38	60.75	10.70	0.55
11 h	56.75	40.88	49.38	69.38	49.97	68.13	60.50	60.38	12.60	0.52
Respiratory rate, breath/min										
-0.5 to 1 h	13.50	30.50	17.63	26.00	26.94	6.25	14.13	6.25	14.47	0.41
1 to 3 h	-7.88	-16.75	-15.63	-13.00	23.52	26.00	-15.63	13.88	14.48	0.02
3 to 5 h	13.75	-2.13	19.13	5.38	-18.85	-8.75	22.75	1.88	8.95	0.12
5 to 7 h	1.13	1.13	-11.63	-3.50	-1.49	-4.38	-7.38	-13.88	10.95	0.63
7 to 9 h[1]	-6.13	3.50	-8.88	12.38	-17.67	10.63	-1.63	6.38	10.90	0.92
9 to 11 h	3.00	-15.50	-0.50	-8.00	-4.17	-9.25	-4.88	-0.38	11.15	0.94

[1]Nursery dietary copper effects ($P = 0.04$).

Table A.6.6. Effects of sow and nursery dietary copper levels (mg/kg) on response of serum cortisol levels to LPS or PBS injection

Challenge:	LPS				PBS					
Sow copper:	20	20	120	120	20	20	120	120		
Nursery copper:	20	220	20	220	20	220	20	220		*P* values
Item Treatment No.:	1	2	3	4	1	2	3	4	SEM	Challenge
No. of observations	4	4	4	4	4	4	4	4		
Cortisol, µg/dL										
0 h	8.02	4.36	6.36	6.33	4.93	3.46	7.31	9.68	1.62	0.94
2 h	15.98	15.06	17.64	22.59	3.99	3.34	5.74	5.92	2.16	< 0.0001
4 h	17.39	19.09	19.22	21.32	2.14	4.85	4.77	6.96	3.23	< 0.0001
6 h	9.38	9.74	9.65	14.51	2.56	3.28	5.05	4.12	2.27	0.00
8 h	8.73	8.00	7.72	10.72	1.80	1.77	3.80	4.56	1.78	0.00
10 h	8.61	7.09	7.27	11.16	1.74	4.10	5.52	5.96	1.93	0.01
12 h	9.95	9.94	8.97	11.92	3.90	4.66	7.35	5.72	1.37	0.00
Cortisol change, µg/dL										
0 to 2 h	7.97	10.71	11.28	16.25	-1.24	-0.12	-1.56	-3.76	1.62	< 0.0001
2 to 4 h	1.41	4.03	1.59	-1.27	-1.54	1.51	-0.97	1.04	1.81	0.28
4 to 6 h	-8.02	-9.35	-9.58	-6.81	0.33	-1.57	0.28	-2.84	2.11	0.00
6 to 8 h	-0.65	-1.74	-1.93	-3.79	-0.80	-1.51	-1.25	0.44	1.48	0.23
8 to 10 h	-0.12	-0.92	-0.45	0.44	-0.06	2.33	1.72	1.40	1.73	0.21
10 to 12 h	1.34	2.85	1.71	0.75	2.68	0.56	1.83	-0.23	1.31	0.58

Table A.6.7. Effects of sow and nursery dietary copper levels (mg/kg) on response of serum interleukin-6 levels to LPS or PBS injection

Challenge:	LPS				PBS					
Sow copper:	20	20	120	120	20	20	120	120		
Nursery copper:	20	220	20	220	20	220	20	220		*P* values
Item Treatment No.:	1	2	3	4	1	2	3	4	SEM	Challenge
No. of observations	4	4	4	4	4	4	4	4		
Interleukin-6, pg/mL										
0 h	12	26	7	7	19	9	38	12	6	0.13
2 h	825	971	700	1069	0	16	68	35	201	< 0.0001
4 h	835	908	399	653	-6	31	65	30	248	0.00
6 h	276	135	155	333	-10	48	71	30	118	0.02
8 h	231	116	127	299	93	124	216	132	62	0.25
10 h	158	76	63	110	28	63	159	76	44	0.52
12 h	147	86	51	116	61	45	148	94	48	0.71
Interleukin-6 change, pg/mL										
0 to 2 h	814	947	693	1062	-20	6	30	23	202	< 0.0001
2 to 4 h	9	-62	-301	-417	-11	16	-3	-5	93	0.01
4 to 6 h	-559	-778	-245	-315	-1	17	6	0	158	0.00
6 to 8 h	-44	2	-28	-33	92	76	145	101	73	0.01
8 to 10 h	-73	-47	-63	-181	-51	-61	-57	-56	39	0.17
10 to 12 h	-11	15	-12	10	29	-17	-11	19	44	0.89

Table A.6.8. Effects of sow and nursery dietary copper levels (mg/kg) on response of serum tumor necrosis factor-alpha levels to LPS or PBS injection

	Challenge:	LPS				PBS					
	Sow copper:	20	20	120	120	20	20	120	120		
	Nursery copper:	20	220	20	220	20	220	20	220		*P* values
Item	Treatment No.:	1	2	3	4	1	2	3	4	SEM	Challenge
No. of observations		4	4	4	4	4	4	4	4		
Tumor necrosis factor-alpha, pg/mL											
0 h		10	3	8	2	3	2	3	3	2	0.14
2 h[1]		2680	7267	1574	3651	4	12	6	13	987	< 0.0001
4 h[1]		157	321	145	281	-5	10	9	14	55	< 0.0001
6 h		46	65	49	101	6	7	6	6	20	0.001
8 h		33	58	44	54	35	13	5	13	13	0.004
10 h		26	16	22	34	4	11	13	8	10	0.01
12 h		23	16	21	29	4	12	8	6	9	0.01
Tumor necrosis factor-alpha change, pg/mL											
0 to 2 h[1]		2670	7264	1567	3649	0	9	3	10	988	< 0.0001
2 to 4 h[1]		-2522	-6951	-1429	-3370	4	-2	2	2	957	< 0.0001
4 to 6 h[1]		-111	-256	-95	-180	10	-2	-3	-8	39	< 0.0001
6 to 8 h		-13	-6	-5	-47	25	6	0	7	18	0.03
8 to 10 h		-7	-42	-23	-20	-30	-2	8	-6	12	0.09
10 to 12 h		-3	1	0	-5	-1	1	-5	-2	5	0.95

[1]Nursery dietary copper effects ($P < 0.05$).

Appendix 7. Pilot study - effects of drying methods on analyzed trace mineral concentrations in different tissues

Objective

Tissue trace mineral concentrations are one of the most frequently used indices in swine research to evaluate trace mineral status of pigs. The harvested tissues normally need to be dried before laboratory analysis to standardize trace mineral concentrations to the same dry matter basis across samples. However, different drying methods have been used in previous trace mineral studies, and oven-drying and freeze-drying were adopted in most studies. The objective of this pilot study was to assess the effects of drying methods on analyzed trace mineral concentrations in different tissues.

Material and Methods

A total of 8 pigs (30.4 ± 1.3 kg BW, 4 males and 4 females) were weighed, stunned by electric shock, and killed by exsanguination. Then the abdominal cavity was opened for collection of the liver, heart, and kidneys. Tissue samples were chopped into small pieces and then ground in a kitchen meat grinder (The butcher shop premium, KRUPS USA, Parsippany, NJ). Subsamples were weighed and dried by desiccation or lyophilization.

Desiccation was accomplished by putting weighed subsamples in forced-air ovens at 55 or 100°C until a constant weight was reached. With regard to lyophilization, subsamples were weighed into plastic containers and placed into the freeze-dryer for 12 to 14 days to a constant weight according to freeze-dryer's operational instructions. Upon completion of drying, all dried samples were finely ground with a coffee grinder. Then all dried and wet tissue samples were digested with nitric acid in a pressurized microwave digestion system and appropriately diluted. The digested solution was used to determine trace mineral

concentrations by atomic absorption spectrophotometry. All the assays were conducted in duplicate. Tissue trace mineral concentrations were then converted to as-is basis, and data were subjected to ANOVA using the GLM procedure in SAS, mean separation was also conducted by using the PDIFF option of SAS.

Results

With regard to dried samples, freeze-drying or oven-drying did not significantly affect trace mineral concentrations of the liver and kidney ($P > 0.09$). However, oven-dried tissue samples had greater concentrations of Cu, Fe, and Zn as compared to those in freeze-dried samples ($P < 0.05$). Meanwhile, the wet (undried) heart samples had greater Fe concentrations but lower Cu and Zn concentrations than dried samples ($P < 0.05$). In addition, mean separation showed the only significant differences were observed on heart Fe and Zn concentrations in samples that were 100°C oven-dried and freeze-dried.

Conclusion

Based on this study, trace mineral concentrations in liver and kidney did not differ between subsamples that were dried by 100°C oven-drying and freeze-drying. However, the length of drying process for 100°C oven-drying is much shorter than that of freeze-drying (2 to 3 d vs. 12 to14 d), and the forced-air oven is more accessible than freeze-dryer; so it was decided to use 100°C oven-drying for all tissue samples in Chapter 3.

Table A.7.1. Effects of drying methods on tissue trace mineral concentrations in liver, kidney, and heart (as-is basis)[1]

		Drying Methods			SEM	*P* values		
Item	UD	FD	OD55	OD100		Treatment	UD vs. D	FD vs. OD
No. of observations	8	8	8	8				
Liver								
Dry matter, %	-	29.05[b]	30.41[a]	27.20[c]	0.27	< 0.0001	-	0.47
Copper, mg/kg	7.05	7.37	6.98	6.88	0.59	0.94	0.97	0.55
Iron, mg/kg	218.35[a]	183.97[b]	196.08[ab]	201.23[ab]	10.28	0.15	0.05	0.25
Manganese, mg/kg	4.21[ab]	3.89[b]	4.29[a]	4.05[ab]	0.13	0.17	0.40	0.09
Zinc, mg/kg	21.95	20.11	20.64	21.10	1.68	0.89	0.50	0.71
Kidney								
Dry matter, %	-	19.23	19.63	18.77	0.41	0.35	-	0.95
Copper, mg/kg	7.88	7.70	7.81	7.65	0.84	0.99	0.87	0.98
Iron, mg/kg	54.60[a]	45.22[b]	43.58[b]	48.50[ab]	3.17	0.09	0.02	0.84
Manganese, mg/kg	1.44	1.46	1.47	1.40	0.07	0.92	0.97	0.83
Zinc, mg/kg	8.57	8.54	9.00	8.52	0.27	0.57	0.72	0.52
Heart								
Dry matter, %	-	20.73[a]	20.80[a]	19.97[b]	0.16	0.002	-	0.12
Copper, mg/kg	3.23[b]	3.28[b]	3.60[a]	3.39[ab]	0.08	0.01	0.04	0.04
Iron, mg/kg	62.13[a]	45.42[c]	50.05[bc]	51.17[b]	1.89	< 0.0001	< 0.0001	0.05
Manganese, mg/kg	0.44	0.40	0.46	0.41	0.05	0.79	0.73	0.62
Zinc, mg/kg	6.52[b]	6.47[b]	6.86[a]	6.93[a]	0.11	0.01	0.07	0.005

[a-c]Least squares means within the same row without a common superscript differ ($P < 0.05$).
[1]UD: undried samples; D: dried samples; FD: freeze-drying; OD55: oven-drying at 55; OD100: oven-drying at 100°C.

Appendix 8. Pilot study – effects of increasing dosages of lipopolysaccharide administration on body weight, vital signs, and serum cytokine levels of growing pigs

Objective

Administration of lipopolysaccharides (LPS) to pigs is extensively used to establish an experimental model of immunological stress with an induced systemic inflammatory response, such as increase the circulating pro-inflammatory cytokines, loss of appetite, lethargy, and fever. The objective of this pilot study was to assess the effects of increasing dosages of LPS administration on body weight, vital signs, and serum cytokines of growing pigs.

Materials and Methods

A total of 9 gilts with initial BW of 24.8 ± 0.4 kg were blocked by BW and assigned to 1 of 3 pens with ancestry balanced across pens; and then experimental treatments (LPS dosages of 25, 50, and 75 µg/kg of BW) were randomly assigned to pens. At 0730 h, 2 pigs from each pen were randomly selected to be intraperitoneally injected with LPS solution at the volume of 5 mL, and the remaining pig was injected with the same volume of phosphate-buffered saline (PBS). The LPS was dissolved in autoclaved PBS solution to make 0.5 mg/mL LPS solution. A volume of 5 mL was prepared for each pig by combining PBS with the appropriate volume of LPS solution. All pigs were allowed to have ad libitum access to a common nursery diet and water during LPS challenge period. Pigs were weighed about 5 min before injection, and 2, 4, 6, 8, and 24 h post-injection; rectal temperature and blood samples were recorded or collected about 5 min before injection and 2, 4, 6, 8 h post-injection; and respiratory rate was recorded about 30 min before injection and 1, 3, 5, 7 h post-injection by counting flank movements for 1 min of resting

pigs. Serum samples were obtained from blood by centrifugation and analyzed for interleukin-6 (IL-6) and tumor necrosis factor-alpha (TNF-α).

Results

Since the pilot study only involved a total of 9 pigs, it was expected to have great variation within treatment and low statistical power. Nevertheless, injection of LPS resulted in greater BW loss, rectal temperature increase, and TNF-α concentration increase within 4 h post-injection compared to those injected with PBS ($P < 0.05$). It suggested that pigs that injected LPS were immunological stimulated. In addition, the pigs that injected with LPS at 50 μg/kg BW had an intermediate response to LPS injection as compared to those injected with 25 or 75 μg/kg BW.

Conclusion

Based on this study, it was decided to use the dosage of 50 μg/kg BW for LPS injection in Chapter 4.

Table A.8.1. Effects of increasing dosages of lipopolysaccharide administration on body weight of growing pigs

		LPS, µg/kg BW				*P* values
Item	PBS	25	50	75	SEM	LPS vs. PBS[1]
No. of observations	3	2	2	2		
Body weight, kg						
0 h	24.62	24.68	25.21	24.84	0.96	0.78
2 h	25.09	24.46	24.83	24.58	0.95	0.66
4 h	25.25	24.29	24.61	24.46	0.93	0.45
6 h	25.40	24.44	24.50	24.38	0.87	0.34
8 h	25.12	24.59	24.42	24.26	0.81	0.45
24 h	25.96	25.06	25.03	23.90	1.11	0.31
Body weight change, g						
0 to 2 h	477[a]	-225[b]	-380[b]	-255[b]	184	0.01
2 to 4 h	160	-165	-225	-120	195	0.17
4 to 6 h	153	155	-110	-85	264	0.57
6 to 8 h	-287	145	-80	-115	188	0.23
8 to 24 h	843	475	615	-360	591	0.38
Cumulative weight change, g						
0 to 2 h	477[a]	-225[b]	-380[b]	-255[b]	184	0.01
0 to 4 h	633[a]	-390[ab]	-610[b]	-375[ab]	349	0.03
0 to 6 h	790	-235	-715	-460	542	0.08
0 to 8 h	507	-90	-795	-575	485	0.11
0 to 24 h	1343	385	-180	-935	1034	0.20

[a, b]Least squares means within the same row without a common superscript differ ($P < 0.05$).
[1]A single degree of freedom contrast was conducted to compare the difference between LPS and PBS injected pigs.

Table A.8.2. Effects of increasing dosages of lipopolysaccharide administration on vital signs of growing pigs

Item	LPS, µg/kg BW				SEM	*P* values
	PBS	25	50	75		LPS vs. PBS[1]
No. of observations	3	2	2	2		
Rectal temperature, °C						
0 h	39.61[a]	39.25[b]	39.78[a]	39.78[a]	0.06	0.86
2 h	39.87[b]	41.06[a]	40.72[ab]	40.56[ab]	0.26	0.02
4 h	40.06[b]	41.33[a]	41.23[a]	40.59[ab]	0.31	0.03
6 h	39.69[b]	40.64[a]	40.59[a]	40.11[ab]	0.16	0.01
8 h	39.80	40.00	39.92	39.87	0.13	0.37
Rectal temperature change, °C						
0 to 2 h	0.26[b]	1.81[a]	0.95[b]	0.78[a]	0.22	0.01
2 to 4 h	0.18[ab]	0.28[ab]	0.50[a]	0.03[b]	0.11	0.48
4 to 6 h	-0.37	-0.70	-0.64	-0.48	0.21	0.34
6 to 8 h	0.11[a]	-0.64[b]	-0.67[b]	-0.25[ab]	0.15	0.01
Respiratory rate, breath/min						
-0.5 h	62.83	48.00	59.75	71.00	8.10	0.72
1 h	58.83[b]	60.00[b]	59.00[b]	98.00[a]	9.81	0.25
3 h	75.50	49.00	39.25	48.50	16.66	0.15
5 h	50.50	45.75	33.50	44.00	8.56	0.34
7 h	46.17	49.75	43.75	50.50	9.43	0.86
Respiratory rate change, breath/min						
-0.5 to 1 h	-4.00	12.00	-0.75	27.00	15.33	0.34
1 to 3 h	16.67[a]	-11.00[ab]	-19.75[ab]	-49.50[b]	18.90	0.08
3 to 5 h	-25.00	-3.25	-5.75	-4.50	11.24	0.14
5 to 7 h	-4.33	4.00	10.25	6.50	9.35	0.30

[a, b]Least squares means within the same row without a common superscript differ ($P < 0.05$).
[1]A single degree of freedom contrast was conducted to compare the difference between LPS and PBS injected pigs.

Table A.8.3. Effects of increasing dosages of lipopolysaccharide administration on serum cytokine levels of growing pigs

		LPS, µg/kg BW				*P* values
Item	PBS	25	50	75	SEM	LPS vs. PBS[1]
No. of observations	3	2	2	2		
Interleukin-6, pg/mL						
0 h	2	2	4	2	2	0.61
2 h	36[b]	48[b]	233[ab]	789[a]	189	0.17
4 h	15[b]	26[b]	103[ab]	249[a]	38	0.04
6 h	12	15	31	356	135	0.43
8 h	19	14	38	137	47	0.42
Interleukin-6 change, pg/mL						
0 to 2 h	34[b]	46[b]	228[ab]	787[a]	189	0.17
2 to 4 h	-21	-22	-129	-540	152	0.24
4 to 6 h	-3	-11	-72	107	102	0.92
6 to 8 h	7	-1	7	-219	89	0.44
Tumor necrosis factor-alpha, pg/mL						
0 h	10	388	57	6	195	0.51
2 h	75[c]	949[bc]	3047[b]	9638[a]	1428	0.003
4 h	28[b]	351[b]	545[b]	2076[a]	199	0.01
6 h	37	510	267	649	235	0.14
8 h	25	433	208	307	181	0.19
Tumor necrosis factor-alpha change, pg/mL						
0 to 2 h	65[c]	561[c]	2990[b]	9632[a]	563	0.002
2 to 4 h	-47	-598	-2502	-7562	665	0.01
4 to 6 h	9	159	-278	-1184	185	0.07
6 to 8 h	-12[a]	-77[ab]	-59[ab]	-343[b]	93	0.19

[a-c]Least squares means within the same row without a common superscript differ ($P < 0.05$).
[1]A single degree of freedom contrast was conducted to compare the difference between LPS and PBS injected pigs.

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Publications

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Honors and Awards

2017. ADAS/ASAS Graduate Student Competition Ph.D. Poster Division. 3rd Place. ADSA/ASAS Midwest Board.

2015. IIC Pinnacle Award. International Ingredients Corporation

2013-2014. Graduate School Academic Year Fellowship. University of Kentucky.

2004-2007. Merit Undergraduate Student. China Agricultural University.

2004-2007. Second Rank Academic Scholarship. China Agricultural University.

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Presentations

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